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STUDIES ON STARCH IN CRUMPETS

by

Ann E. Weatherall

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH
IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE
OF **Doctor of Philosophy**

Department of Food Science

EDMONTON, ALBERTA
Spring 1986

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ISBN 0-315-30248-8

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DEGREE FOR WHICH THESIS WAS PRESENTED **Doctor of Philosophy**

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ABSTRACT

Starches were isolated from two commercial flours used in the production of crumpets. It was found that starch from the hard wheat flour was more damaged. Starch from the soft wheat flour exhibited the greater swelling power while solubilities of the two flours were essentially equal. The fact that viscosity of a dilute suspension of starch from the soft wheat flour was greater than viscosity of a suspension of the other starch indicated that viscosity development under these conditions was more a factor of swelling of the granules than the existence of a filamentous exudate.

The two starches exhibited the same temperature and heat of gelatinization as measured by differential scanning calorimetry (DSC). Addition of emulsifiers, monoglyceride (MG) and sodium stearoyl-2-lactylate (SSL) to model batters had no effect on temperature or heat of gelatinization. When SSL and MG were added to batter in dispersion form they promoted complexation of amylose and lipid while in powder form complexation increase over a control sample was either minimal or non-existent. Addition of a dispersion of SSL at a 0.45% level based on flour weight did not result in more complexation than when the level used was 0.3% of flour weight. Even when complexation was promoted as determined by DSC and X-ray diffraction methods there was no noticeable effect on either crystallization of starch or firming of crumpets. Starch crystallization and crumpet firming

proceeded at a higher rate and to a greater degree during storage at 5°C than during storage at 25°C.

Use of Avrami kinetics was found helpful in describing starch crystallization in the crumpets during storage at 5°C but less so during storage at 25°C. This method was of little use to describe changes in firmness as measured by compression techniques.

Crumpet structure was devoid of a continuous protein network but was made up of tightly-packed deformed starch granules with protein masses dispersed throughout.

ACKNOWLEDGEMENTS

I would like to thank my supervisor, Dr. B. Ooraikul, and the other members of the committee for their help and advice during the course of this project.

Financial support was provided in the form of a Canadian Commonwealth Scholarship and a University of Alberta Dissertation Fellowship and is gratefully acknowledged.

The collaboration of Dr. F. Longstaffe, Department of Geology, and the technical assistance of Ms. D. Carid in the determination of X-ray diffraction patterns of crumpets are greatly appreciated.

Thanks go to Ms. M. Cerrone for technical assistance with scanning electron microscopy and differential scanning calorimetry. Crumpets were kindly donated by Mr. R. Forrest of Forcrest Foods Ltd., Calgary.

I would like to thank Mr. L. Steele for typing and proofreading the manuscript and Dr. F. Scriven for additional proofreading. The efforts of Mr. A. Bates and Mr. G. Choudhury in preparation of the figures made my work load lighter and are appreciated.

Warm thanks go to Willie for inviting me to share her home during the last hectic few weeks. I thank my family for their support and, last but not least, special thanks go to Luis for his encouragement, help and love throughout my Ph.D. program.

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1. INTRODUCTION

Crumpets are a traditional English baked product which are becoming popular in other parts of the world such as Australia and New Zealand (Loney and Crawford, 1976), Western Canada and parts of the United States (Forrest, 1985). In comparison with bread, the volume of sales of this product is small (Forrest, 1985), which accounts for the paucity of published material dealing with crumpets as found by Loney and Crawford (1976). However there has been a renewed interest in this product since it is an example of those baked goods which respond well to gas packaging (Ooraikul, 1982).

Loney and Crawford (1976) stated that a good quality crumpet is well risen with an even cell structure, not blinded at the top, and of a tender texture. These researchers undertook a study to determine the most suitable flour type for the production of crumpets in New Zealand and, by use of somewhat subjective test methods, found that crumpets of high quality were obtained by using a finely-milled low protein flour. The local manufacturer in Alberta found that he obtained optimum results from his processing operation by blending equal amounts, by weight, of two flours of differing protein content.

Gas packaging and reformulation techniques have led to a much extended shelf-life for crumpets (Smith *et al.*, 1983, 1984) with consequent benefits to the producer such as improved plant utilization, reduction in returns to the manufacturer and the possibility of opening up new markets farther afield. The major drawback to the longer product shelf-life was found to be changes occurring which may be described by the term "staling".

The major ingredients of crumpets and bread are similar but the relative proportions of these ingredients, method of manufacture, packaging and patterns of consumption differ considerably. It was decided that an investigation of starch in crumpets would provide an insight into the formation of structure of the crumpet and the effect on the product of prolonged storage. Since addition of emulsifiers is a widely used method of reducing deleterious effects of storage, the possible use of two of these agents was investigated.

The purpose of this study was:

1. To characterise the flours and starches used by the local manufacturer in crumpet production.
2. To study gelatinization of starch during baking and the effect on it of emulsifier addition.
3. To study the microstructure of crumpets and batters.
4. To conduct storage trials at two temperatures and quantify the changes occurring.
5. To assess the suitability of application of Avrami kinetics to describe staling in crumpets.

2. LITERATURE REVIEW

2.1 Flour and Starch

Hardness of wheat has a significant effect during the milling process and the manner in which hard wheat fractures is different from that of soft wheat (MacRitchie, 1980). Flours milled from soft wheats tend to have less damaged starch than those from hard wheats. MacRitchie (1980) found that the particle size distribution in flours from soft and hard wheats is different, with a greater proportion of small particles measuring less than 25 µm (i.e. material in the free starch granule and protein fragment range) being present in soft wheat flour (35.9% of flour) than in hard wheat flour (15.8% of flour).

The microscopic appearance of starches from different botanical species is usually so characteristic that identification can be made in this way (Seidemann, 1966; D'Appolonia *et al.*, 1971). The wheat kernel contains two types of starch granules: the large A-granules, which are formed first, and small B-granules, which are formed at a later stage of development (Badenhuizen, 1969; Baruch *et al.*, 1979; Buttrose, 1963; Evers, 1969, 1970, 1971; Evers *et al.*, 1974). A-granules are lenticular in shape, most having a diameter of 20-35 µm (Kerr, 1950), although Hoyer (1911) reported an upper limit of 55 µm, while B-granules are spherical with diameters in the range of 2-10-µm (Kerr, 1950). MacMasters and Waggle (1963) pointed out that there are also granules in the size range intermediate between A- and B-granules. The assertion of a unimodal granule size distribution by Bathgate and Palmer (1972) has not gained acceptance.

A-granules were found to have an equatorial groove (Burhans and Clapp, 1942; Sandstedt, 1955), which was first observed in a scanning electron micrograph by Evers (1969). The contribution of B-granules to total starch weight and volume for a variety of cereals has been reviewed by Hanssen *et al.* (1953) who concluded that their presence had been badly underestimated because of poor starch recovery during extraction. D'Appolonia *et al.* (1971) summarized earlier work and concluded that B-granules contribute less than 10% by weight. However, Evers (1973) used microsieving to show that B-granules make up 30% of starch weight; this was later confirmed by Evers and Lindley (1977).

The wheat starch granule is largely made up of the two polyglucan molecules, amylose and amylopectin, although it has been claimed that intermediate material, some of which is linear and some of which is lightly branched, is always present (Banks and Greenwood, 1975; Greenwood, 1976) at a level of about 5-10% of most cereal and root starches. These constituents are organized into a radially anisotropic, semi-crystalline unit (Blanshard, 1978), with the anisotropy being responsible for the distinctive Maltese cross, seen when the granules are observed in polarized light, while the semi-crystallinity, evident when studied by X-ray diffraction, reflects the presence of both ordered and amorphous regions. There is no sharp demarcation between the crystalline and amorphous phases of starch granules, and it is generally believed that some or all of the starch molecular chains run continuously from one phase to another (Senti and Dimler, 1960).

2.2 Swelling and Gelatinization of the Starch

Gelatinization of starch is the basis for many types of food production. The wheat starch granule has been more extensively studied than any other cereal (Blanshard, 1978) but, despite the many studies on its gelatinization, this phenomenon is still not fully understood (Lund, 1984).

Wheat starch is generally considered to be insoluble in water before gelatinization (Knight, 1965), although swelling of the granule does occur due to water uptake (Kerr, 1950; Lund, 1984). Several authors have stated that this initial swelling is reversible (Kerr, 1950; Hellman *et al.*, 1952; Schoch, 1965; Banks and Greenwood, 1975; Greenwood, 1976; Lund, 1984) but Dengate (1983) has questioned this assertion, noting that Gough and Pybus (1971) suggested that some degree of irreversibility of swelling may be introduced after prolonged exposure to warm water. Dengate (1983) concluded that there is no clear evidence for the thermal reversibility of pregelatinization swelling.

Glicksman (1969) gave a traditional explanation of starch gelatinization; the constituent molecules in the starch granules being held together by hydrogen bonding and the behaviour of starch being mainly governed by the affinity of hydroxyl groups in one molecule for those in another. The "initial gelatinization temperature" is the temperature at which the hydrogen bonding forces of the starch granule in aqueous suspension weaken to the point where water can be absorbed (Olkku and Rha, 1978). At this point the granules swell tangentially and simultaneously lose their birefringence, which is taken to indicate irreversible loss of orderly molecular orientation (Dengate, 1983). Gelatinization phenomena start at the hilum or botanical centre of the granules and spread rapidly to the periphery (Dengate, 1983), occurring initially in the amorphous areas since the hydrogen bonding is weakest in those areas (Lund, 1984). As the temperature is increased, intermolecular hydrogen bonds which maintain the structural identity of the granule continue to be disrupted.

Leach *et al.* (1959) and Schoch (1964) investigated the swelling pattern of starches. Swelling power was measured by heating a dilute starch suspension from 60 to 95°C and, at 5 C° increments, determining the weight of sedimented paste compared with the dry starch weight, corrected for soluble material. Solubility was determined simultaneously by withdrawing aliquots of the supernatant and drying them to allow determination of the soluble material exuded by the granules.

The two stage swelling shown by wheat starch has been attributed by Leach *et al*. (1959) to two sets of hydrogen bonds that relax at different temperatures. The small amount of swelling between 60 and 70°C involved the disruption of weakly bound or readily accessible amorphous sites, and a second rapid swelling around 80 to 90°C involved the disruption of more strongly bound or less accessible crystalline sites.

Gelatinization of starch was studied using proton magnetic resonance (PMR) techniques by Jaska (1971). When a 40% potato starch slurry was heated, water mobility increased suddenly at 52°C, the onset of gelatinization and solubilization. A 20% starch slurry showed a sudden increase in line width (i.e. decrease in water mobility) at that temperature, caused by the sudden hydration upon gelatinization decreasing the water mobility with respect to that of free water. The average water mobility depends upon the ratio of water molecules in close association with starch to the total water and on the mobility of the starch molecules with which the water molecules are associated. At both concentrations, above 55°C the water mobility slowly increased due to increasing mobility of the macromolecules. Jaska (1971) considered that, at temperatures of 63 to 76°C, the starch granule had swollen to the point where its crystallinity had been destroyed and all the soluble starch was in solution inside the granule before exudation, indicated by the increase of mobility detected by PMR.

After gelatinization, viscosity increases markedly, regardless of starch concentration. This viscosity increase was formerly ascribed to granules imbibing water during swelling and so increasing the probability of their contact with each other (Miller *et al.*, 1973). Rha (1975) thought this a plausible explanation for the increase in consistency, since this increase is a measure of hydrodynamic volume and of the work required to move granules past each other as they are expanding. However, the importance of exudation was shown by Miller *et al.* (1973) who found that the maximum viscosity of wheat starch suspension occurred after most of the granule swelling had ceased. The increase in viscosity was shown by light and scanning electron microscopy to be caused mainly by exudate released from the granules and appearing as a filamentous network among them. Further evidence was obtained when Miller *et al.* (1973) added sodium stearyl fumarate (0.1% of starch weight) to a starch suspension before heating in the amylograph. Granule swelling and viscosity development were normal up to 70°C but both reached a maximum at this temperature while viscosity of an untreated suspension continued to increase. Even though the treated granules were larger at 95°C than the normal granules, the viscosity of the suspension was much less. Miller *et al.* (1973) found that exudation of amylose had been prevented by the addition of the emulsifier and thus concluded that increased viscosity during starch pasting is due to exudation of a filamentous network of amylose rather than to granule swelling.

Application of solid-state light-scattering techniques to the starch granule has demonstrated that it may be treated as a polymer spherulite (Banks and Greenwood, 1975), with the proviso that its shape and nucleation centre are governed by the plastid in which it is formed so that spherical symmetry cannot always be attained. With this background, starch gelatinization has been considered as a melting process (Blanshard, 1979). Lelièvre (1973) used the theories of polymer chemistry, developed by Flory (1953), which related the melting temperature of a crystalline polymer to the concentration of diluent in the system, to treat the gelatinization of starch as the melting of a homogeneous polymer. He examined the relationship between gelatinization temperature of the starch granule under equilibrium conditions and the water content of a dispersion of starch granules (Lelièvre, 1973). By this model of gelatinization as a melting phenomenon, the presence of water and other additives would be expected to lower the melting point (gelatinization temperature) of the "pure" (i.e. dry) starch (Evans and Haisman, 1982) and there have been many reports of the corollary, that reducing the water content tends to raise the gelatinization temperature (Hellman *et al.*, 1954; Collison and Chilton, 1974; Priestley, 1975). It has been shown by the use of differential scanning calorimetry that the effect on gelatinization temperature of reducing the water content is somewhat more complicated. Donovan (1979) showed that, when the ratio of water to starch was less than about 2:1 (g/g dry starch), the gelatinization temperature range widened. As the water content was reduced further, the endotherm split into two peaks, the upper end of the range moving to higher temperatures. Eventually, the lower peak disappeared while the upper peak continued to move upwards (Donovan, 1979; Ghiasi *et al.*, 1982c; Eliasson, 1980). Donovan (1979) fitted these results to the standard equations for the melting of polymers in a diluent system, assuming that the upper limit of the high temperature endotherm represented the true melting point of the system. He suggested that the splitting of the endotherm was due to the swelling of the amorphous regions of the starch granule "stripping" starch polymer chains from the crystallites and effectively tearing them apart (Donovan, 1979; Biliaderis *et al.*, 1980; French, 1984). Under limited water conditions this process becomes superseded by conventional "melting", which is manifested by the high temperature endotherm.

The Flory equations (1953) describe the effects on the system of diluents in terms of their volume fractions and the various interaction coefficients. Evans and Haisman (1982) have challenged the use of volume fractions calculated using the whole system as the basis, which presupposes that the starch-water system is homogeneous.

They studied the gelatinization of potato starch and applied the Flory equation using volume fractions based on granule composition.

Gelatinization of starch in water occurs over a range of temperatures characteristic of the type of starch and usually of the order of 10 C° (Evans and Haisman, 1982; French, 1984). However, individual granules gelatinize over about a 1 C° temperature range. Thus, the broad range observed for total gelatinization of the starch is due to differences in susceptibility amongst the granules. Evans and Haisman (1982) suggested that each starch granule has its own population of crystallites covering a broad range of stabilities and that the narrow gelatinization range of a single granule is due to a highly cooperative process of crystallite melting followed by absorption of water, which lowers the melting point of further crystallites. They have used similar reasoning to explain the appearance of a single high temperature endotherm at low water contents (Evans and Haisman, 1982) and claim that their postulate more fully accounts for the observed phenomena than does that of Donovan (1979), which attributes the endotherm shift to stresses induced by the swelling of amorphous regions of the starch granules.

Marchant and Blanshard (1978) postulated three constituent processes for starch gelatinization:

1. the diffusion of water into the starch granules;
2. the disappearance of the birefringence on heating -- a

hydration facilitated "melting" process;

3. a swelling which is also a diffusion type process.

Blanshard (1978) reported that total exchange of water between a starch granule and the environment at ambient temperature occurs in about one second. Thus, based on this observation and the temperature dependence of gelatinization, it is not the diffusion process that is responsible for starch gelatinization. Blanshard (1978) reviewed two hypotheses to explain the behaviour of starch granules during gelatinization and stated that the hypothesis that it is a semi-cooperative rather than cooperative process is the more satisfactory. In this way, the focus is shifted from the granule as a whole to the crystallites within the granule, each with slightly different energy characteristics.

Olkku and Rha (1978) summarized the steps of gelatinization based on changes in characteristics of starch granules during and after heating in an aqueous medium:

1. granules hydrate and swell to several times their original size;
2. granules lose their birefringence;
3. clarity of the mixture increases;
4. marked rapid increase in consistency occurs and reaches a maximum;
5. linear molecules dissolve and diffuse from ruptured granules;
6. upon cooling, a uniformly dispersed matrix forms a gel or

paste-like mass.

Several factors have an influence on the gelatinization of starch, among them moisture content, amylases, lipids, ions and salts, and pH (Dengate, 1983; Lund, 1984). Lelièvre (1976) extended the Flory equation from two to three components to relate the composition of starch/water/solute mixtures with the melting of the crystalline polymer, starch.

Wootton and Bamunuarachchi (1980) studied the effect of sodium chloride and sucrose on starch gelatinization. Sodium chloride affected the initial, peak and completion temperatures and also the size of the temperature range of gelatinization. Increasing the level of sucrose decreased the heat of gelatinization and raised the peak temperature while the initial and final temperatures of the transition remained constant. Bean and Osman (1959) concluded that sucrose inhibited the swelling of starch granules, increased the onset temperature, lowered maximum consistency, reduced the thinning of the paste after completion of gelatinization, and decreased the gel strength upon cooling. However, Olkku and Rha (1978) considered this conclusion to be only partially true for wheat starch.

Several explanations for the restrictive effect of sucrose on the gelatinization process (Wootton and Bamunuarachchi, 1980) have been proposed, including competition between starch and sucrose for available water, sucrose inhibition of granular hydration, and sucrose-starch interactions (Lund, 1984). Since a linear relationship between heat of gelatinization and sucrose level in the aqueous phase was not found (Wootton and Bamunuarachchi, 1980), the actual mechanism by which sucrose affects starch gelatinization is not simply a lowering of the available water.

While starch will not gelatinize on boiling in concentrated sodium sulphate solution, it will gelatinize in sodium iodide at room temperature (Gough and Pybus, 1973). It is generally accepted that, if starch is exposed to a series of anions, each having the same cation, the resulting changes in the gelatinization temperature will occur in the same order as the occurrence of the anions in the lyotropic series (Medcalf and Gilles, 1966; Banks and Greenwood, 1975), although the situation is more complex in terms of the mode of swelling (Gough and Pybus, 1973). In general it has been found that the more strongly hydrated ions compete for water with starch molecules, thus reducing swelling (Dengate, 1983). Work by Evans and Haisman (1982) on potato starch, and by Oosten (1982, 1983) suggests that this view may be simplistic. Oosten's hypothesis is that starch acts as a weak acid ion exchanger and that cations tend to protect and to stabilize the granule structure while anions perform the gelatinizing by rupturing hydrogen bonds.

The influence of solutes on gelatinization appears to be very rapid, thus as ionic and nonionic constituents dissolve, they readily interact with the starch granule, altering the gelatinization characteristics.

2.3 Starch in Baked Products

Baked products are extremely complex systems since they involve a large number of ingredients. During the course of baking, heat induces physical and chemical changes in the components of the batter or dough system which yield a stable structure with subjectively desirable flavour, aroma and texture characteristics.

Greenwood (1976) was of the opinion that the role of the starch granule in baking had been underestimated and stated that it probably contributes more to the structure and texture than had been generally recognized. Gelatinization of native starch grains embedded in the glutenous matrix of bread dough is apparently essential to the formation of a porous elastic crumb (Rotsch, 1954; Sandstedt, 1961). Substitute fillers such as glass beads (Rotsch, 1954), pregelatinized starch (Kim and de Ruiter, 1968) and native high amylose starch grains which show only limited gelatinization (Kim and de Ruiter, 1968) have all proved to be unsatisfactory. It has been suggested that in gelatinizing during cooking the starch takes up water from the surrounding gluten which thus becomes dehydrated and semi-rigid (Sandstedt, 1961; Shellenberger *et al.*, 1966; Medcalf, 1968).

Microscopic techniques are valuable research tools for studying baked products. The first observations of the microscopic structure of bread are generally considered to be those published by Verschaffelt in 1912. Since then, many investigators have used transmission light microscopy (TLM), transmission electron microscopy (TEM) and scanning electron microscopy (SEM) to elucidate the microstructure of baked goods.

Due to the great commercial value of bread, much work has been carried out on dough and bread microstructure. The typical dough has a gluten network giving it integrity and elasticity which is a result of the input of work during the breadmaking process (Angold, 1979). Moss (1974) concluded that a different interpretation should be placed on the significance of the gluten film to that quoted by such workers as Farrand (1972) that the aim of mixing was to spread the gluten to form a continuous matrix, and that a film of gluten, stabilized by addition of oxidising agents, covered the surface of the starch granules. According to Moss (1974), in an optimally developed dough, the gluten forms a network of interconnected sheets that surround many of the starch granules but do not completely envelop them. Some starch granules are trapped between the gluten sheets but have little or no associated gluten. A film of gluten may be formed when the dough has either been overmixed or mixed with an excess of cysteine (Moss, 1974). Angold (1979) gave a micrograph of wheat flour dough showing that starch granules may be held together by three forms of gluten, namely sheets which line the gas bubble, strands and fine filaments which appear to adhere to the surface of the granules.

The state of organization of starch granules as shown by optical and scanning electron microscopy varies depending on the type of bake product (Greenwood, 1976). In Scottish shortbread, the granules retain their natural characteristics and are merely swollen or partly gelatinized, while in biscuits (cookies) granules can vary from being in the swollen to being in the disrupted state. Granules in cakes are always gelatinized and often disrupted, whereas in bread the whole spectrum from gelatinized to enzymatically degraded granules is apparent. Wafers are unique in that their structure is a matrix of dispersed starch material with no visible organized structure (Stevens, 1976).

Sandstedt *et al.* (1954) found that starch granules in a dough were oriented by the pull of the expanding gas bubble during fermentation. After gelatinization during baking, further expansion of gas bubbles caused a distortion of the flexible granules, they did not disintegrate but retained their structural identity (Sandstedt *et al.*, 1954). Dennett and Sterling (1979) found that gelatinizing wheat starch granules seemed to be more responsive to the tensions in baking dough than other starches that they tested. The wheat starch granules lined up in more closely parallel array in the crumb cell walls.

The large wheat starch granules are discoid with a median plane of weakness (Melchior and Feuerberg, 1954; Evers, 1971; Gallant and Guilbot, 1973). Melchior and Feuerberg (1954) stated that, when they are gelatinized, radial contraction and limited expansion of the granules lead to splitting and to the formation of two flat, narrow, plate-like disks.

Hoseney *et al.* (1978) stated that the major role of starch in cakes is to act as a water-sink and to set the structures in the baking process. Howard *et al.* (1968) showed that intact granular starch was essential in the thermal-setting process of cake baking. The thermal-setting stage is that time at which the batter changes from a fluid, aerated emulsion to a solid, porous structure that is stable on removal from the oven (Howard *et al.*, 1968). At this point, the water absorption properties of the starch granules actually control the final physical characteristics of the baked cake although other components can compete with the starch for the water present in the system.

Gordon *et al.* (1979) used SEM to study the structure of "lean" experimental batters and cakes in which pure wheat starch replaced the cake flour at levels from 0 to 50% on a weight/weight basis. They found that the starch was suspended as lumps in the batter matrix and noted no difference in the batter appearance over the tested range of flour and starch ratios. The crumb structure of the finished cakes was found to be similar at all levels of starch substitution tested, but there were differences in the starch granules and the cake matrix. The state of the starch granules appeared to be dependent on their position within the cake and was attributed by Gordon *et al*. (1979) to differences in temperature and hydration gradients during baking.

Francis and Groves (1962) used the light microscope in a study of bread, cake and biscuits, using gelatin embedding and being mindful of the risks of artefact generation during the hydration of baked goods. They concluded that in all baked goods, from biscuits to cakes, there exists a gluten network even though the network may be very tenuous when compared to that seen in bread dough. Exceptions to this are "short sweet" types of biscuit, where the structure is made up by fat and sugar bridges. They also found that in a wafer (made from batter baked rapidly between hot plates at a temperature of about 200°C) there was no structure in the strict sense and only a random and formless mass in which air was entrapped in strands of denatured protein intermingled with completely gelatinized starch. Stevens (1976) used SEM to study the structure of wafer biscuits. He found that the starch component of the flour was completely gelatinized by the baking process, with no evidence whatever of granular starch structure. It was also noted that a batter consisting of starch and water produced a wafer similar to the normal product and thus it was concluded that [illegible]arch was the continuous phase and structure-forming component.

In "high ratio" cakes (i.e. cakes with a higher proportion of liquid and sugar than normally used), Francis and Groves (1962) found a diffuse protein network to be the basis of the structure but far less dense and interconnected than seen in breadcrumb. The starch present appeared to be more formless and more gelatinized than that seen in breadcrumb and to play a considerable part in the crumb structure. They found it impossible to say whether the starch or the protein was the structure-forming material.

2.4 Staling

Bread and related products are important throughout the world with high nutritional and economic impact. Figures given in 1971 (Ponte, 1971) and quoted in 1975 (Maga, 1975) and 1981 (Kulp and Ponte, 1981) stated that an average of 8% of the bread produced in the United States was returned to the bakery because it was unsaleable due to non-microbial deterioration. This spoilage is termed "staling" and has been defined by Bechtel *et al.* (1953) as indicating decreasing consumer acceptance of bakery products by changes in crumb other than those resulting from the action of spoilage organisms. Kulp and Ponte (1981) suggested that the term staling as defined by Bechtel *et al.* (1953) be used in a generic sense and that specific terms such as crumb firming, crust staling and organoleptic staling be used to describe individual components of the staling process. Many review articles, primarily covering bread staling, have appeared (e.g. Elton, 1969; Willhoft, 1973a, 1973b; Zobel, 1973; Maga, 1975; Knightly, 1977; D'Appolonia and Morad, 1981; Kulp and Ponte, 1981).

Even though research into the staling phenomenon has been taking place since 1852, when Boussingault demonstrated that it was not due specifically to an overall loss of moisture, all the questions have still not been answered. Boussingault (1852) was also the first to publish evidence that stale bread could be refreshened by heating to above 60°C. Von Bibra (1861) showed that stale rye bread required at least 30% moisture content before it could be refreshened by heating. In 1876, Horsford proposed that staling was due to exchange of water from starch to gluten, thus leaving the particles hard and causing the bread to firm. Boutroux (1897) was the first to suggest that staling involved the formation of a derivative of starch, a view which was modified by Lindet (1902) who proposed that a less soluble form of starch was produced, terming the reaction "retrogradation". Research into bread staling was carried out from 1912 to 1916 by Katz and described by him in 1928 (Katz, 1928). He attributed firming to starch retrogradation, proposing that both amylose and amylopectin were affected. Ostwald (1919) agreed that starch is the chief factor in staling of bread but ascribed the change in the starch to an internal aggregation and dehydration with consequent loss of water by syneresis. Fuller (1938) proposed a reduction in hydration capacity and a change in the proportion of α- and β-amylose to be important in explaining staling.

Schoch and French (1947) found that most amylose retrogradation takes place during baking and subsequent cooling and therefore attributed the staling process to the heat-reversible aggregation of amylopectin. They also noted that dissociation of retrograded amylose can only be accomplished after heating to 125°C, while stale bread can be refreshened at lower temperatures. These results were supported by those of Kim and D'Appolonia (1977c). As recently as 1983 (Russell, 1983b) amylopectin has been described as being primarily responsible for the bread staling phenomenon.

Erlander and Erlander (1969) postulated that ageing of crumb is due to aggregation of amylose and amylopectin, a process which is inhibited by complex formation of the starch polymers with lipids and proteins. In addition to starch, Willhoft and co-workers (Willhoft, 1971a,b; Breaden and Willhoft, 1971; Cross *et al.*, 1971) concluded that the gluten fraction played a role in staling. They considered the firming process in starch to be due to the development of regions of crystallinity within the granules while the gluten phase rigidified by transfer of water to the starch. Willhoft (1971a) used a specially designed cell to separate starch and protein fractions and observed moisture migration from protein to starch. Zobel (1973) pointed out, however, that Willhoft may have limited his experimental approach to studying only the gluten-starch interface.

Cornford *et al.* (1964) investigated the relationship between elastic modulus of crumb and time and temperature range from -1 to 32°C, confirming that the rate of bread staling has a negative temperature coefficient. They also found that all the breads tested tended to firm to the same firmness value but that the rate of firming depended on storage temperature.

A physical process with molecules coming into a more ordered arrangement than originally (i.e. crystallization) is thus implicated as being the principal factor involved in crumb firmness. Further supporting evidence for the importance of starch crystallization in staling has been obtained by using the Avrami concept. In a series of papers, Avrami (1939, 1940, 1941) developed a theory of the kinetics of phase change that was later simplified by Evans (1945) and Morgan (1955), who expressed the change in crystallinity of a system by the equation:

$$\theta = \exp(-kt^n) \qquad \text{[Eq 2.1]}$$

where θ represents the fraction of a crystallizing material not yet crystallized at time t, k is a rate constant, and n is the Avrami exponent, which is a combined function of the number of dimensions in which crystal growth takes place and the order of the time dependence of the nucleation process. The Avrami equation can be used to describe the staling process if it is due to a crystallization change- involving the transformation of an amorphous starch component into an ordered crystalline state. For application to crumb firming data, the further assumption of a direct relationship between the rate of crumb firming and rate of crystallization must be made.

Using crumb modulus measurements, Cornford *et al.* (1964) applied the Avrami equation as follows:

$$\theta = (E_L - E_t)/(E_L - E_0) = \exp(-kt^n) \qquad \text{[Eq 2.2]}$$

The term E_L in the equation represents the limiting modulus, E_t the modulus at time t and E_0 that at zero time. For the purpose of calculation, it is assumed that no crystalline starch is present when bread comes from the oven and that the starch is entirely crystalline when the limiting modulus is reached. That the Avrami equation is applicable to crumb staling was further confirmed by employing differential thermal analysis (DTA) (Axford and Colwell, 1967; McIver *et al.*, 1968; Colwell *et al.*, 1969). Axford and Colwell (1967) observed an endothermic peak, absent in fresh crumb, developing during storage in a manner very similar to that of increase in crumb firmness as reported by Cornford *et al.* (1964). The process causing the peak proved to be reversible since it was absent during a repeat run on the same sample of initially stale bread. This observation agrees with the phenomenon of refreshening stale bread by heating to around 60°C. Differential scanning calorimetry (DSC) is a more suitable technique than DTA when measurement of endotherms is involved and it has been used recently in the application of the Avrami analysis to ageing of starch gels and baked goods (Longton and LeGrys, 1981; Fearn and Russell, 1982; Eliasson, 1983b; Russell, 1983a, 1983b). Use of computers has facilitated the simultaneous estimation of the three unknown parameters of the Avrami equation, thus obviating the former practice of experimental determination of the limiting value of the measured response.

The granule structure of wheat starch shows an X-ray diffraction pattern, termed A, which disappears on gelatinization. Katz (1928) demonstrated that the X-ray patterns of starch pastes and breads change during ageing. Freshly-baked bread, without added emulsifiers, shows only V-crystallinity due to amylose reacting with the naturally occurring fatty acids in the starch granules. The remaining pattern is an amorphous halo due to the gelatinized starch. The characteristic diffraction line in the V-pattern is that at 4.4 Å, the intensity of which may be used as a measure of complex formation (Zobel, 1973). Crystallization of starch during ageing of starch or breadcrumb has been followed by changes in the intensity of the 5.2 Å spacing of the B-structure that is typical of retrograded starch (Zobel, 1973; Pisesookbunterng *et al.*, 1983). The molecular conformation of the retrograded starch is an extended one, contrasting with the tight helix of the V-structure. During staling the intensity of the V-pattern remains virtually unchanged. When heated under moist conditions, aged samples of breadcrumb or starch gels give mainly amorphous X-ray diffraction patterns (Zobel, 1973). Hence, heating causes a melting or reversion of the starch crystallization that occurred with ageing.

X-ray diffraction patterns of bread supplemented with α-amylase have shown that they exhibited greater crystallinity than an unsupplemented control (Dragsdorf and Varriano-Marston, 1980). However, the enzyme-supplemented (especially with bacterial α-amylase) breads were softer than the control. Although an increase in starch crystallinity during storage of the control bread paralleled increased firming, this was not the case with enzyme-supplemented breads. It was therefore concluded (Dragsdorf and Varriano-Marston, 1980) that increases in starch crystallinity are not synonymous with bread firming. Zobel and Senti (1959) suggested an explanation for the increase in the starch crystallinity in α-amylase-supplemented breads as being due to the more rapid movement of degraded components into lattice position facilitated by their lower molecular weights. New crystallites can grow out from nuclei that are potentially present in the amorphous structure wherever chain segments happen to be parallel in lattice positions.

Using a quantitative X-ray analysis of starch crystallinity in bread crumb, Wright (1971) showed the rate constant, k, for crystallization at 4 and 21°C to be essentially the same as that obtained from modulus and DTA data on starch gels and bread crumbs by using Avrami-type functions.

Presumably due to the smaller volume of sales involved, the staling of cakes and other baked products has received much less attention than that of bread. However, many articles on the subject have been published, including those by Pence and Standridge (1958), Kulp *et al.* (1959), Hodge *et al.* (1978), Maxwell and Zobel (1978) and Guy (1983). Hodge *et al.* (1978) gave the definition of cake staling as "the increase in crumb firmness and harsher eating quality which occurs when cake is stored". The Avrami analysis has been applied to staling of Madeira slab cake (Guy, 1983). Firmness data obtained from crustless cakes stored with insignificant moisture loss were used to provide a measure of the intrinsic firming process of the cell wall constituents. The Avrami exponent was found by graphical means to approximately equal unity, which agrees with results obtained from studies on bread and wheat starch gels (Cornford *et al.*, 1964; McIver *et al.*, 1968; Colwell *et al.*, 1969; Kim and D'Appolonia, 1977b). The rate constant was found to be highest at 5°C and to fall as the storage temperature rose from 5 to 21°C. However, the firming rate of cake crumb began to level off between 21 and 30°C and to increase again at 40°C.

Hodge (1977) unsuccessfully attempted to refreshen stale cake crumb by heating and thus came to the conclusion that the crystallization of starch did not play an important role in cake staling. However, Guy (1983) found that model gel systems could be refreshened by heating at 100 to 105°C and concluded that previous attempts to refreshen crumb in whole cakes (Hodge, 1977) probably failed because the moisture level in the crumb had fallen due to its migration to the crust.

Colwell *et al.* (1969) found that the rate constant (determined using the Avrami analysis) for firming of breadcrumb at 40°C was greater than that for starch crystallization and therefore postulated that the latter process might only be part of the firming mechanism at temperatures greater than about 30°C. Guy (1983) concluded that, although at subambient temperatures the crystallization of starch is probably the dominant process in cake staling, at higher temperatures the mechanism depends even less on starch crystallization than in the case of bread. The secondary process implicated at temperatures above 30°C has a positive temperature coefficient and may be related to moisture diffusion between the protein and the swollen starch granules.

Cloke *et al.* (1983) used DSC to measure enthalpies associated with starch transformations in a lean cake batter and in the crumb of cakes, both fresh and after storage at 4°C for 4 days. They found no endotherm activity due to starch transformation in either cake and thus suggested that crystallization did not occur during storage.

MacRitchie (1980) pointed out the apparent anomaly that starch, generally assumed to be the dispersed -phased in baked products, should be held responsible for determining their rheological properties. Starch is a dispersed phase in dough but the gelatinization processes which occur during baking cause some of the starch to be expelled from the granules. The granules swell and distort, forming contacts and, in some cases, partially coalesce, which results in a continuous structure in the baked product.

The many variables associated with staling may be used as indices to measure the progress of the ageing process. Herz (1965) listed the following changes occurring: changes in taste and aroma, increases in the hardness of crumb, increases in the opacity of crumb, increases of crumbliness of crumb, increases in starch crystallinity of crumb, decreases in absorptive capacity of crumb, decreases in susceptibility of crumb to attack by β-amylase enzyme, and decreases in soluble starch content.

Over the years, many methods have been devised to measure rate and degree of staling. These methods are based on physical, chemical and sensory properties and have been reviewed by Maga (1975). Perhaps the most common group of methods is that employing compression of the sample. One of the official AACC procedures (AACC, 1982) involves compression with the Baker Compressimeter and is based on the determination of the force applied to produce a uniform compression of the sample. Researchers who have used compression methods to follow staling include Platt and Powers (1940), Noznick *et al.* (1946), Cornford *et al.* (1964), Bishop and Wren (1971), Mälkki *et al.* (1978), Chamberlain *et al.* (1981), Neukom and Walter (1981), Roewe *et al.* (1982) and Guy (1983).

It has been stated that any instrumental measure of texture must ultimately relate to evaluations made by humans (Kramer, 1972) and, to this end, work has been carried out to determine correlations between sensory and instrumental techniques. Axford *et al.* (1968) compared crumb moduli for a series of loaves of bread of different ages and specific volumes with an evaluation of their staleness by a taste panel. The panel rating of the staleness and the logarithm of the measured firmness of the bread were highly significantly correlated. Bashford and Hartung (1976) concluded that a rheological test could be standardized to be a valid predictor of bread freshness as perceived by the consumer. Brady and Mayer (1985) measured four textural parameters of rye and French breads using a sensory panel and compression techniques and calculated correlation coefficients. They found that results from instrumental measurements indicated significant differences between samples which were not picked up by sensory evaluation. Two explanations were suggested for this discrepancy:

1. the panel and the instrument were not measuring the same parameter, or
2. the instrument was more sensitive to slight differences in individual parameters of the samples than were the panelists.

It has been found experimentally (Axford *et al.*, 1968) that high specific volume bread loaves have a lower initial crumb modulus and show a smaller change in modulus with time on staling than those of low specific volume. It was concluded that both the rate and extent of staling decreased as loaf specific volume increased. Thus a loaf of unknown specific volume with a given crumb modulus could be either fresh low specific volume bread or stale high specific volume bread. Guy and Wren (1968) developed a method whereby breadcrumb was centrifuged to obtain a gas-free pellet of cell-wall material which could be compressed to give firmness values independent of specific volume. This method has been used recently in following staling of Madeira slab cake (Guy, 1983).

Organoleptically perceived staleness may be argued to be of paramount importance since staleness is a phenomenon that is perceived by humans. However, Fearn and Russell (1982) advocated the use of DSC to follow crystallization of starch during staling as a method which may lead to a fundamental insight into the molecular changes occurring during staling.

Pentosans have been shown to retard starch retrogradation, the effect exerted by water-insoluble pentosans being more pronounced than that by water-soluble pentosans (Kim and D'Appolonia, 1977a). Kim and D'Appolonia (1977d) showed that pentosans also decreased the staling rate of bread stored at 21°C.

It has been found that spraying cakes with a level of 3% by weight of ethanol appeared to arrest the staling process as measured by crumb firmness (Hodge *et al.*, 1978). Russell (1983c) painted loaves of bread with ethanol which resulted in a reduction in the rate of development of the endotherm associated with starch crystallization and a reduction in the rate of increase in crumb modulus during storage. He did not establish the site of action of the ethanol.

The improving effect of lipids on loaf volume is negatively related to bread staling (D'Appolonia and Morad, 1981). Pomeranz *et al.* (1966) reported that nonpolar lipids reduced crumb firmness only slightly while polar lipids were very effective. Russell (1983b) found that glyceryl monostearate had a definite effect in reducing both the rate of breadcrumb firming and the rate of starch crystallization in the crumb. Pisesookbunterng *et al.* (1983) observed that addition of emulsifiers to bread was helpful in restoring the original freshness of stale crumb by heating.

2.5 Emulsifiers

Emulsifiers or substances with emulsifying properties occur in the majority of foodstuffs and their raw materials. For example, mono- and diglycerides are intermediates in the biosynthesis and biodegradation of both plant and animal fats (Schuster and Adams, 1984). Griffin and Lynch (1968) stated that the naturally present emulsifiers play a major role in the growth processes of foods and assist in the metabolism that converts them into sources of energy. As food additives, emulsifiers exhibit food-specific effects in addition to their classical surface active effects.

Data for food use of emulsifiers in the United States in 1981 (Schuster and Adams, 1984) showed that mono- and diglycerides were most heavily used (total usage, 200.2 x 10^6 lb; 116.0 x 10^6 lb in bread and rolls), followed by lactylic esters of fatty acids, sodium and calcium stearoyl lactylate (total usage, 30.5 x 10^6 lb; 27.0 x 10^6 lb in bread and rolls).

When dissolved or dispersed in a liquid, emulsifiers are preferentially adsorbed on an interface resulting in a number of physicochemical or chemical alterations. Such a compound contains at least one moiety with affinity for strongly polar substances and at least one moiety with affinity for non-polar substances. A criterion for the solubility of emulsifiers is a hydrophile-lipophile equilibrium (Harris, 1933). This idea was quantified by Griffin (1949), who assigned emulsifiers a number from 1 to 20 in a hydrophilic-lipophilic balance (HLB) scale. Lipophilic emulsifiers are given lower HLB numbers than those with hydrophilic character, with the turning point between lipophilic and hydrophilic properties being 10 on this scale. The HLB values of nonionic emulsifiers can be calculated using the formulae developed by Griffin (1954), on the basis that the HLB value is a function of the ratio (by weight) of the hydrophilic moiety to the total molecule of the surface active substance.

Heusch (1970) modified the formulae to expand the HLB scale to 40 in order to make possible calculation of HLB values for ionic compounds. Commercial monoglycerides have an HLB of 5.0 or less (MacDonald, 1968), while sodium stearoyl-2-lactylate has an HLB of 21.0 (Lorenz and Dilsaver, 1982).

A monoglyceride (MG) is an ester of a fatty acid with glycerol. Esterification of either of the end carbons produces a 1-, 3- or α-MG, while esterification of the central carbon results in a 2- or β-MG. The MGs are commonly sold as a mixture of mono- and diglycerides (MDG) (Van Haften, 1979). However, the actual functional emulsifier is only the MG portion of the mixture, with the α-MG being significantly more functional than the β-MG. Maximum MG concentrations of ca. 65% are feasible with existing commercial manufacturing processes (Van Haften, 1979). To obtain higher concentrations the mono- and diglyceride mixture is distilled under high vacuum to give a product of ca. 92-95% MG.

Sodium stearoyl-2-lactylate (SSL) and its calcium counterpart (CSL) are usually manufactured by reacting stearic acid with lactic acid at 180 to 200°C in the presence of the desired base with the water of reaction being removed by inert gas sparge (Van Haften, 1979).

2.6 Crystal Form

Polymorphism is defined as the property of crystalline modifications that depend upon pressure and temperature conditions (Schuster and Adams, 1984). It has been demonstrated by various workers that the fats exhibit distinct polymorphic behaviour, depending on their composition and pretreatment (Lutton and Jackson, 1948, 1967; Lutton, 1950; Singleton and Vicknair, 1951; Chapman, 1956, 1960; Kuhrt *et al*., 1963; Skau, 1973). When the melt of a long-chained unbranched aliphatic triglyceride is cooled, the hexagonal α-form, which generally has the lowest melting point of all the modifications, is formed. When the α-form is cooled to -50 to -70°C, a sub-α-form occurs having an orthorhombic or triclinic crystal lattice which is quite similar to that of the α-form. However, the symmetry is altered in that the free rotation of the hydrocarbon chains possible in the α-form is not allowed in the sub-α-form. The transition from α-form to sub-α-form is reversible (Schuster and Adams, 1984). When a triglyceride in the α-form is warmed to around its melting point, an exothermal conversion into a more stable β'- or β-form takes place. If the β'-form is warmed further, a rapid transition to the stable β-form occurs, these conversions being irreversible. The β-form has a higher melting point and lower energy content than the α-form. Emulsifiers used in foods exhibit varying degrees of similarity to the triglycerides and thus varying degrees of polymorphism.

The interaction of emulsifiers with starch has been the subject of many investigations which have focussed on their influence on the rate of gelatinization, the gelatinization temperature, the peak viscosity, the gel strength and the formation of complexes with starch.

Krog (1973) found that MG increased the gelatinization temperature of wheat starch more than did SSL and CSL, while none had an appreciable effect on corn or potato starch. The gelatinization temperature and the peak viscosity of wheat starch were affected by the pH and ion concentration. Ghiasi *et al.* (1982a) reported that SSL inhibits the swelling and solubility of wheat starch up to 85°C while MG ($C_{16:0}/C_{18:0}$) inhibits them up to 120°C. These emulsifiers also reduced the water absorption of the starch in the amylograph during heating (Ghiasi *et al.*, 1982d).

Eliasson (1983b) found that addition of SSL to a gluten-starch-water mixture decreased the heat of gelatinization and increased the peak temperature of the transition. She used two different levels of SSL and noted that the gelatinization temperature increase was greater for the higher level while the heat of transition was similar for both SSL levels. These alterations in the gelatinization process indicate a complex reaction mechanism.

Schoch and Williams (1944) reported that the straight chain fraction of corn starch formed a precipitate with fatty acids. Mikus *et al.* (1946) ruled out surface adsorption and hydrogen bonding and, by means of X-ray structure analysis, implicated a helical form of amylose with the fatty acid bound inside. Rundle and co-workers (Rundle and Baldwin, 1943; Rundle and French, 1943a,b) had previously presented evidence that starch chains possessed a helical configuration in the starch-iodine complex. Schoch (1965) reasoned that amylose reacts instantly with MG to form a helical complex, implying that the MG permeates the granule. Longley and Miller (1971) also concluded that the complex was formed in the interior of the starch granules.

Osman (1972) found that amylopectin exhibited only a slight tendency to form inclusion compounds and concluded that the formation of complexes by a starch fraction in a foodstuff system depends upon the concentration of the inclusion molecules, their molecular structure, the number and size of the functional groups and the temperature of the mixture.

Several authors (Lagendijk and Pennings, 1970; Hoover and Hadziyev, 1981; Krog, 1981) have shown that the length and unsaturation of the fatty acid chain affects the ability of an MG to combine with starch. Osman (1972) also stated that double bonds and large hydrophilic moieties generally reduce their complex-forming capacity. This may be explained by the fact that a saturated MG has a straight chain of about 4 Å outer diameter (Lagendijk and Pennings, 1970) and fits into the amylose helix which had been found to complex with a wide range of complexing agents with cross-sectional diameter of 4.5-6.0 Å (Takeo and Kuge, 1969). The chains of unsaturated MG molecules are bent (Boekenoogen, 1967) owing to the double bonds and, due to steric hindrance, may not enter the amylose helix as easily. Krog (1971) has shown that, for a homologous series of saturated MGs containing from C_8 to C_{22} fatty acids, the complexing efficiency (on a weight basis) was highest for the C_{14} derivative, and that it decreased in a regular fashion for shorter and longer chain compounds. However, Riisom *et al.* (1984) have shown that in some cases cis-unsaturated MGs will precipitate amylose more than saturated compounds and thus emphasized the importance of the physical state of the MGs in amylose complexing.

Van Lonkhuysen and Blankenstijn (1974) found that even at 30°C a portion of MG (27% C_{16} and 73% C_{18}) is irreversibly bound to starch granules in a suspension. The amount of MG bound increases considerably during gelatinization and reaches an equilibrium at 90°C. They concluded that at 30°C each starch granule is surrounded by a micelle-like MG layer up to 38 molecules thick, held together by hydrogen bonds and accounted for the increase to 90°C by helical inclusion compounds.

De Stefanis *et al.* (1977) showed that the emulsifier-starch interaction is pH dependent, that the greatest quantities of emulsifier are bound in the pH range 3.95-7.35, and that not only amylose but also amylopectin participates in the reaction. Ghiasi *et al.* (1982b) used X-ray structure analysis to show that SSL and MG form strong complexes with wheat starch at 60 to 80°C. The thermal stability varies, with SSL-starch complexes being cleaved at 95°C while MG-starch complexes could be detected up to 120°C.

Practically all authors agree that the emulsifiers in amylose complexes are located within the α-helix and present evidence that the complex is only slightly attacked by either β-amylase (Ghiasi *et al.*, 1982a; Kim and Robinson, 1979), α-amylase (Van Lonkhuysen and Blankenstijn, 1976) or a combination of the two (Acker and Brauner-Glaesner, 1982). Treatment with ether does not increase starch hydrolysis by β-amylase (Kim and Robinson, 1979).

Carlson *et al.* (1979) found the conformation of the hydrocarbon chains within the amylose helix to be ordered as in the crystalline state, with the polar group located outside the helix. The hydrocarbon chain is surrounded by three turns of the α-helix (Lagendijk and Pennings, 1970; Carlson *et al.*, 1979). According to Kim and Robinson (1979), amylose exists in the α-helical form before complexation, in agreement with the views of Bear and French (1941), Rundle and French (1943b), Rundle *et al.* (1944) and Mikus *et al.* (1946), who concluded that no substantial change in conformation occurs during formation of the complex. Banks and Greenwood (1971), however, maintain that the amylose exists as a statistically random coil with no helix component and that helix formation occurs only in the presence of complex-forming substances (Schoch, 1965; Acker and Brauner-Glaesner, 1982).

Bourne *et al.* (1959) and Krog and Nybo-Jensen (1970) revealed no complex formation between emulsifiers and amylopectin while Lagendijk and Pennings (1970) observed weak interaction between MG and amylopectin. Osman (1972) concluded that this lower complex-forming capacity is due to the limited ability of amylopectin to form a helix.

Wren (1968) reported that the α-crystalline form of MG is very active since the emulsifier molecules are arranged with their polar groups oriented towards the water (Larsson, 1967). In their study, Krog and Nybo-Jensen (1970) found that the α-crystalline gel of MG gave the highest complexing index with amylose. The complex forming capacity was more strongly dependent on physical state at 31°C than at 60°C.

Differential scanning calorimetry (DSC) has been used to study the emulsifier-starch interaction since the endotherm obtained in the temperature range between 90 and 110°C when heating starch suspensions is due to melting of an amylose-lipid complex (Eberstein *et al.*, 1980; Kugimiya *et al.*, 1980). A calorimetric method for determining the amylose content of starches based on the formation and melting of amylose-lysolecithin complex was proposed by Kugimiya and Donovan (1981).

Eliasson (1983b) added SSL in dispersion form to a gluten:starch:water (0.2:1:0.9) mixture and found that the enthalpy of the amylose-lipid complex disordering transition was proportional to the amount of SSL added. Similar results were obtained by Russell (1983b) who found that inclusion of glyceryl monostearate in the gel state as a bread dough constituent led to an increase in the amylose-lipid endotherm.

When emulsifiers are used in breadmaking they generally aid in one or both of two possible functions, namely crumb softening and dough strengthening (Tenney, 1978). The breadcrumb softening effect of emulsifiers is closely related to their ability to complex starch (Krog, 1981; Krog and Nybo-Jensen, 1970; Russell, 1983b). When MGs are added to a bread dough in the form of β-crystalline hydrates, the thin β crystals adsorb to the surface of starch granules during dough mixing. During the baking process, the β crystals of MGs transform first into the α-crystalline state and then into a liquid crystalline phase in which form the MGs are active in amylose complex formation. This may take place at temperatures of about 50°C, before the starch granules begin to gelatinize. The swelling of starch granules in bread is therefore delayed and the amount of free amylose formed is diminished by the reaction with emulsifiers, resulting in a softer crumb structure (Krog, 1981).

While crumb softeners act mainly due to their reaction with starch, dough conditioners act by interaction with the flour protein. SSL is an example of an emulsifier which has a dual effect since it is able to interact with both starch and protein (Birnbaum, 1977). Krog (1981) stated that emulsifiers that are most effective as dough conditioners are all able to form lamellar mesophases or gel structures in water at dough mixing temperatures. He therefore suggested that their functionality is related to their ability to form ordered structures similar to biological membranes in the water phase of the dough.

3. EXPERIMENTAL

3.1 Flour and Starch Properties

3.1.1 Flours used in the study

Flours produced by Ogilvie Mills Ltd., Montréal, Québec and currently used in commercial production of crumpets by Forcrest Foods Ltd., Calgary, Alberta, were supplied by the latter company. The two flours used were Five Roses Enriched and Primrose Special Cake. Compositional analyses as supplied by the miller are given in Table 3.1.

Five Roses Flour is a bleached, enriched top patent flour milled from Canadian hard red spring wheat, bleached with benzoyl peroxide, containing low levels of fungal α-amylase and with 8 ppm potassium bromate added as a late acting maturing agent. Primrose Special Cake Flour is a high ratio soft wheat flour of extra fine granulation, bleached with benzoyl peroxide and treated with chlorine.

3.1.2 Isolation of starch from flour

Isolation of starch from flour was accomplished by a dough washing technique, being a modified version of that described by Shogren *et al.* (1969). A slurry was prepared by mixing 300 g flour with 600 ml distilled water and then centrifuged for 20 min at 1,000 g in a Beckman J-21 centrifuge fitted with a JA-14 fixed angle rotor (Beckman Instruments Inc., Palo Alta, CA, USA). Starch was washed out

Table 3.1 Compositional analyses of commercial flours used in the study.

	Percentage of Flour'	
Component	Five Roses	Primrose Special Cake
Moisture	14.00	14.00
Ash	0.39	0.34
Protein	11.50	8.00
Fat	1.00	0.80
Fibre	0.30	0.15
Carbohydrates	72.81	76.71

' Data supplied by miller.

from the centrifugate using distilled water and the resulting suspension was filtered through 109 and 196 mesh silk in sequence. The filtrate was combined with the supernatant from the centrifuge operation and recentrifuged at 6,500 g for 30 min. The central part of the starch layer was recovered, being the prime starch, while the tailings, pentosans and other components were discarded. The recovered starch was slurried with distilled water and filtered under suction through Whatman No. 4 filter paper (W.&R. Balston Ltd., England). Surface lipids were removed by washing three times with 50 ml aliquots of 95% ethanol and once with 50 ml of ethyl ether. The starch was partially dried by three washes of 50 ml of acetone and the resulting filter cake was spread on filter paper and air dried at room temperature for 24 h.

3.1.3 Moisture determination of flour and starch

Moisture contents of flours and starches were determined according to the AACC method 44-40 (AACC, 1982) which entailed heating at 100°C in a vacuum oven for 5 h. Loss in weight was reported as moisture content.

3.1.4 Scanning electron microscopy (SEM) of starches and flours

Starch and flour samples were of sufficiently low moisture content to undergo SEM without further-treatment. Starch granules or flour particles were sprinkled onto aluminum stubs which had been coated with silver paint. Excess particles were blown off the stub and those adhering to the stub were coated with 20 nm thickness of gold, then examined at an accelerating potential of 10 kV on a Stereoscan 250 scanning electron microscope (Cambridge Instruments Ltd., Cambridge, U.K.).

3.1.5 Determination of damaged starch

The level of damaged starch in the flours and in the isolated prime starches was determined using the procedure described in detail by Williams and Fegol (1969). This method is a modification of a test based on the observation that the amylose present in mechanically damaged starch granules was more rapidly extracted by saturated ammonium sulphate solution than was that in sound starch granules (Hampel, 1952).

Damaged starch was quantified in terms of the colour developed on addition of iodine to an extract made by treating a 1 g flour or starch sample with 25 ml of a strong solution of sodium sulphate containing 15% formamide and 0.2% sulphosalicylic acid for 15 min. The results were recorded as absorbance of the test solution at 555 nm multiplied by a factor of 10 and were the average of duplicate determinations.

3.1.6 Determination of protein in the isolated starch fractions

The protein content of the starch isolates was determined using a macro-Kjeldahl procedure. Duplicate samples were processed.

3.1.7 Swelling power and solubility of starches

3.1.7.1 Swelling power and solubility determination

The procedures used have been described by Schoch (1964a) and involved the stirring of a dilute starch suspension at 200 rpm for a given time at known temperature. The stirrer was then removed and rinsed off into the vessel with sufficient distilled water to bring the total amount of water present to 200.0 g, including the original moisture in the starch sample. After centrifugation at 2,200 rpm in a swinging bucket rotor (Model S2K, International Equipment Co., Needham Hts., MA, USA), the supernatant was removed, a 50 ml aliquot being used to determine the solubility, and the weight of the swollen starch granules remaining in the vessel was measured.

In the present study, triplicate starch samples of 1.0 g were run at temperatures of 60 to 97.5°C inclusive at intervals of about 5 C°. Solubles in the supernatant were determined by evaporating to dryness followed by vacuum drying. The following equations were employed to calculate the results:

$$X = 400\ W_1/W_2 \qquad \text{[Eq 3.1]}$$

where:

X = percentage of solubles on dry basis

W_1 = mass of soluble starch in 50 ml aliquot of supernatant (g)

W_2 = mass of starch sample on dry basis (g)

and

$$Z = 100\ W_3 \ / \ (100-X)\ W_2 \qquad \text{[Eq 3.2]}$$

where:

Z = swelling power

W_3 = mass of sedimented paste (g)

3.1.7.2 Amylose content of the supernatant

The amylose content of the supernatant obtained from the solubility and swelling power determination was estimated by using the rapid colorimetric procedure developed and described by Williams *et al.* (1970) and used more recently by Kim and D'Appolonia (1977c). Duplicate 25 ml aliquots of supernatant from each run were analysed. Absorbance of the iodine-amylose complex was read on a Beckman DU-8 UV-Visible Spectrophotometer (Beckman Instruments Inc., Irvine, CA, USA) at 625 nm against a reference consisting of all the components except the supernatant aliquot which was replaced by distilled water.

Williams *et al.* (1970) have found the use of a standard curve based on highly purified potato amylose satisfactory for use with amylose from other sources. A regression equation was obtained from data given by those authors to calculate the amount of amylose present in solution:

$$y = 0.2469 + 14.607x \qquad \text{[Eq 3.3]}$$

where:

y = mass of amylose in solution (mg)

x = absorbance of the test solution at 625 nm

The square of the sample correlation coefficient (r^2) for Equation 3.3 was 0.998. Results were also calculated in terms of amylose as a percentage of solubles present in the supernatant.

3.1.8 Viscosity of dilute flour and starch suspensions

3.1.8.1 Preparation of suspension

The method of preparation was a modified version of that described by Evans and Haisman (1979). Duplicate slurries of concentration of 2% (w/w, dry basis) were made using distilled water. Flour or starch and water at 50°C were mixed in an Erlenmeyer flask using a magnetic stirrer bar. The stoppered flask was then placed in a boiling water bath until the slurry temperature reached 90°C, at which time it was transferred to a water bath at 90°C where it was held stationary for 20 min. Flask and contents were then held at 35°C for 30 min prior to measurement of viscosity.

3.1.8.2 Measurement of viscosity

Viscosity measurements were made in a Haake Rotovisco RV3, fitted with an NV sensor system and MK50 measuring system (Gebrüder Haake, Karlsruhe, Germany). The method of viscosity measurement was based on that described by Muller

(1973) in that rotation of the cylinder was increased in steps of 10 rpm from 0 rpm to 200 rpm and then decreased in similar steps to 0 rpm. Each speed of rotation was held for 30 s to allow stabilization of the shear stress. Muller (1973) recommended that the motor be turned off between application of the different shear rates but, due to the long response time of the viscometer used in the present study, this practice was not followed. Thus, in this experiment, the rate of change of shear rate was not known but was assumed to be similar for each sample run. Viscosity of triplicate samples from each slurry was determined.

3.1.9 Mineral analysis of flours and starches

Mineral analysis of flours and starches was carried out at the Alberta Agriculture Feed Laboratory, Edmonton, Alberta, using the Inductively Coupled Plasma Technique. Samples were prepared for analysis by digesting a 1 g sample with 10 ml of a mixture of nitric and perchloric acids in the ratio of 2:1 (v/v) for about 20 min at low temperature until charring was complete. Digestion was continued at higher temperature until the digest was essentially colourless. After cooling, the volume was made up to 50 ml with deionized water and this solution was used for mineral analysis. Results were in µg of mineral/g of flour or starch (wet basis) and were the mean of determinations on duplicate samples.

3.1.10 Differential scanning calorimetery (DSC)

All DSC studies during the present work were carried out using a DuPont 990 Thermal Analyzer with a 910 DSC cell base (DuPont Co., Wilmington, DE, USA) and set on time base. The calibration coefficient, E, of the cell was determined using a known mass of sapphire by the continuous method, thus allowing calculation of E at any temperature. However, since the instrument was always run in the 'calibrated' mode, E was essentially constant with temperature for any given constant set of experimental conditions. At least three replicate determinations were made for all procedures involving DSC.

Figure 3.1 shows a generalised DSC thermogram with the characteristic quantities marked. Transition temperatures were obtained by operating the second recorder pen on a temperature scale which was calibrated from the baseline in mV/cm. The conversion to °C was made by using thermocouple tables (Biliaderis *et al.*, 1980). The hatched area of Figure 3.1 is proportional to the heat flow associated with the transition and was measured by means of a planimeter. Heat of transition was calculated according to Equation 3.4:

$$\Delta H = A\ (60\ B\ E\ \Delta qs)\ /\ m\ C \qquad \text{[Eq 3.4]}$$

where:

ΔH = heat of transition (J/g)

A = peak area (cm^2)

B = time base setting (min/cm)

E = cell calibration coefficient (mW/mV)

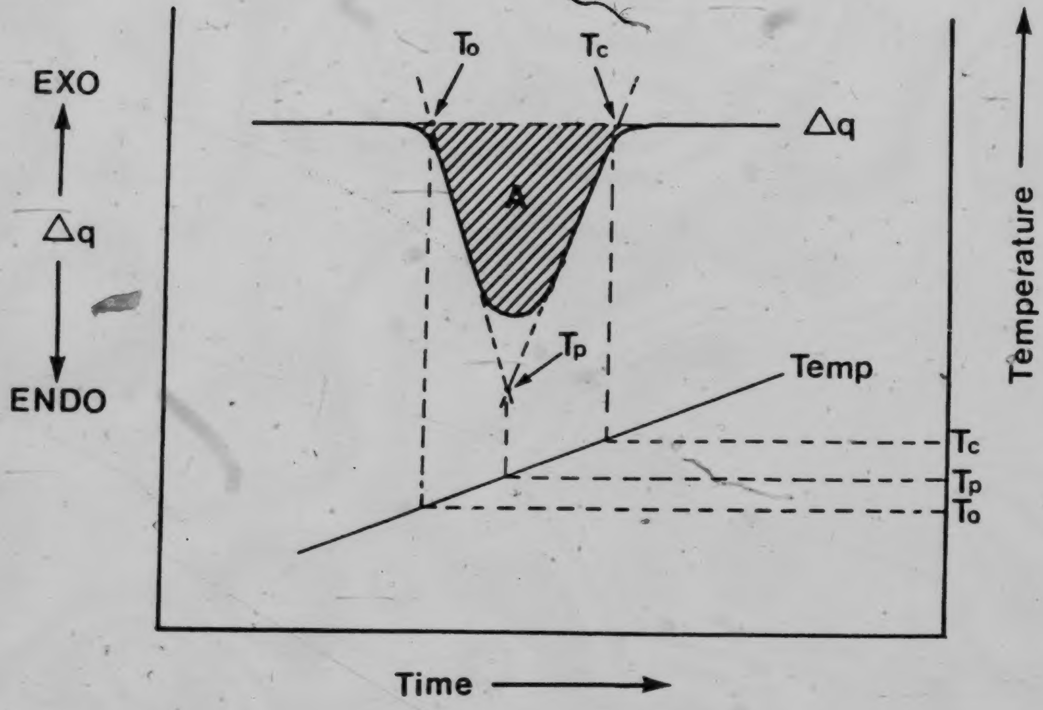

To
Tc
EXO
Δq
Δq
ENDO
A
Tp
Temp
Tc
Tp
To
Temperature
Time

Figure 3.1 Generalized DSC endotherm. T_o, onset temperature; T_p, peak temperature; T_c, completion temperature; A, peak area.

Δqs = Y axis range setting (mV/cm)

m = sample mass (mg)

C = weight fraction of starch if ΔH is in J/g starch

= 1 if ΔH is in J/g sample

3.1.10.1 DSC studies of flours and starches

DSC studies of the flours and starches were carried out to determine the temperature range over which the starch gelatinized and the heat of gelatinization. A slurry was prepared to the required water:starch (dry basis) ratio of 2:1. This ratio can be considered to promote starch gelatinization in a manner that is not restricted by lack of water (Colonna *et al.*, 1981). The vessel containing the slurry was covered to prevent dehydration during the required manipulations. Approximately 15 mg of slurry were transferred to a preweighed aluminum sample pan using a Pasteur pipette and accurately weighed. The pan was hermetically sealed using the provided sealing instrument and reweighed to check for water evaporation during the weighing process. The sample pan prepared thus was left overnight at room temperature to allow equilibration and reweighed prior to DSC analysis. Samples were heated from 10 to 100°C at 10 C°/min using an empty pan and lid as reference.

3.2 Manufacture and Packaging of Crumpets

3.2.1 Formulation and manufacture

3.2.1.1 Standard formulation

The standard crumpet formulation is shown in Table 3.2.

3.2.1.2 Manufacturing procedure in the factory

Water at 43°C was metered into a high speed mixer, the two flours added and mixing started. The remaining dry ingredients were mixed together with a little water up to the required weight and added into the mixer followed by the vinegar and high speed mixing was carried out for one minute. The batter was pumped to a reservoir over a moulded conveyor system heated from below to 200°C and was dispensed into the moulds. Crumpets remained on the conveyor for about 4 min after which time they were cooked through. They were removed and placed in a cold room at 10°C for about 40 min to cool to about 15°C prior to gas packaging in packs of 8 in an atmosphere of 60% carbon dioxide and 40% nitrogen. Packaging was accomplished by using a continuous form-fill-seal vacuum/gas packaging device (Kramer and Grebe Tiromat, Waterloo, Ont.). The packaging material used was 12/75 polyester/polyethylene top web and 100/100 nylon/polyethylene bottom web. Factory produced goods arrived at the laboratory about 18 h after production, having been transported by private car and stored overnight at room temperature.

Table 3.2 Standard batter formula.

Ingredient	Quantity[1] (kg)	Percent	Percent (flour basis)
Cake flour[2]	20.0	20.2	50.00
Strong flour[3]	20.0	20.2	50.00
Salt	0.96	0.98	2.40
Vinegar[4]	1.36	1.38	3.40
Sorbic acid	0.34	0.34	0.85
Monocalcium phosphate	0.09	0.09	0.23
Glucono-δ-lactone	0.43	0.43	1.08
Sodium bicarbonate	0.31	0.32	0.78
Water[5]	55.4	56.04	138.50

[1] Quantities for factory batch.
[2] Primrose Special Cake Flour, Ogilvie Mills Ltd., Montréal, Qué.
[3] Five Roses Flour, Ogilvie Mills Ltd., Montréal, Qué.
[4] 5% acetic acid.
[5] At 43°C.

3.2.2 Headspace gas analysis

Initial headspace gases (CO_2 and N_2) in several packs chosen at random from the consignment were checked on receipt. Analysis was accomplished using a Varian Aerograph Chromatograph Model 90-P (Varian Associates, Palo Alto, CA, USA) equipped with a TCD. A two column system adapted from Murakami (1959) was used to obtain a complete chromatogram of the components in the gas mixture (including any O_2 which may have entered the pack through a poor seal or a puncture) from a single injection. The operating conditions are summarized in Table 3.3. Helium was used as the carrier gas at a flow rate of 50 ml/min. Samples were prepared for analysis by attaching a small amount of RTV silicon sealant (Canadian G.E., Toronto, Ont.) onto each test package. This enabled small amounts of headspace gas (1,000 µl) to be withdrawn using a gas-tight syringe (Hamilton Co., Reno, NV, USA) without damaging the integrity of the packaging material. Percentage by volume of gases was calculated from standard curves of CO_2 and N_2 constructed by measuring peak heights obtained from known volumes of pure gases.

3.2.3 Temperature profile of crumpets during cooking

Crumpets were prepared in the laboratory using the standard formulation and ingredients supplied by the commercial manufacturer. Quantities were scaled down to a 1 kg batch with mixing being accomplished by a free-standing domestic food mixer at top speed for 3 min. Crumpets were

Table 3.3 Operating parameters for analysis by gas chromatography.

Column	Dimensions and Packing Materials	Operating Temperatures (°C)	Function
1	46 x 0.318 cm Carbosieve S60/80	Column ---- 65 Injector -- 125 Detector -- 125	Separation of CO_2
2	90 x 0.318 cm Molecular sieve 5A60/80	Column ---- Ambient Injector -- 125 Detector -- 125	Separation of O_2 and N_2

cooked within metal rings of height 2.5 cm on a Garland Griddle Model No. E22 18G (Russell Food Equipment Ltd., Edmonton, Alta.) that had been modified by addition of an aluminum cover which served to retain heat and steam over the cooking crumpets thus matching more closely the factory conditions.

The temperature profile of a crumpet during cooking was obtained by fitting a metal ring with three thermocouples as shown in Figure 3.2. Temperature was recorded using a Fluke 2240B Datalogger (John Fluke Mfg. Co. Inc., Mountlake Terrace, WA, USA) at 30 s intervals over a period of 5 min.

3.2.4 Emulsifiers

Two different commercial food grade emulsifiers were used at levels chosen because they were found in preliminary trials to give good machinability in the factory operation.

Emplex

Sodium stearoyl-2-lactylate (SSL) was obtained from C.J. Patterson Co., Kansas City, MO, USA, as a cream-coloured powder.

Monomul-D

Distilled monoglyceride (MG) was obtained from J.R. Short Milling Co., IL, USA, as a free-flowing powder. Fatty acid composition as given by the supplier is shown in Table 3.4.

Table 3.4 Fatty acid composition of Monomul-D.

Fatty Acid		%
Myristic	$C_{14:0}$	0.1
Palmitic	$C_{16:0}$	11.0
Stearic	$C_{18:0}$	44.0
Oleic	$C_{18:1}$	42.0
Linoleic	$C_{18:2}$	2.9

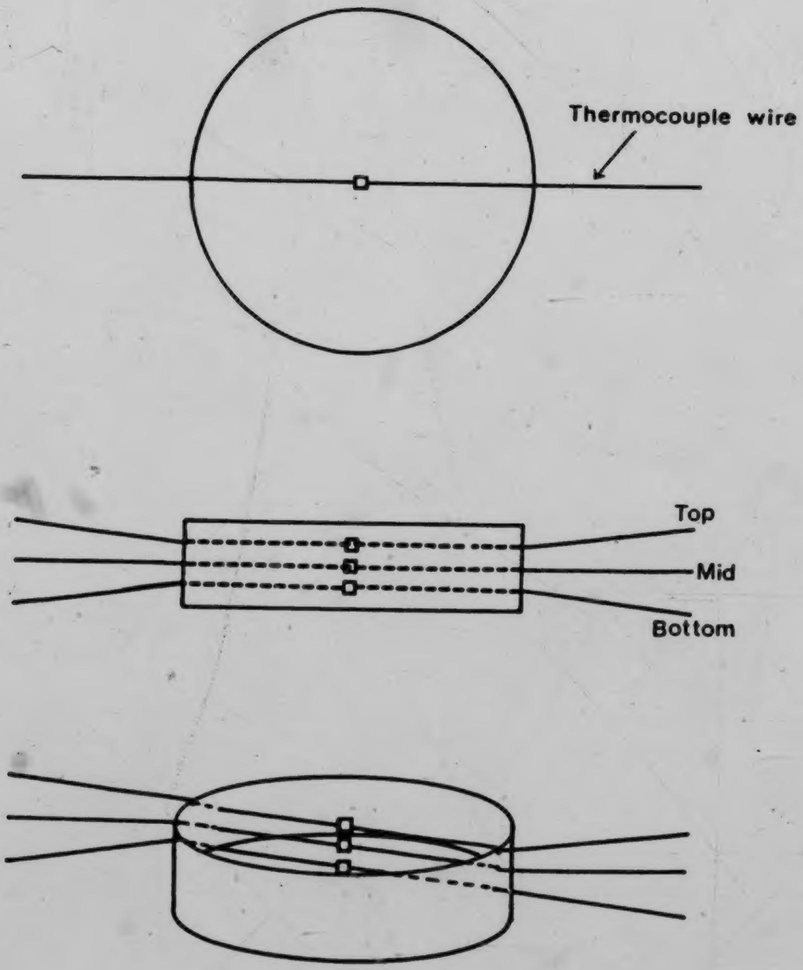

Thermocouple wire
Top
Mid
Bottom

Figure 3.2 Arrangement for measuring temperature profile of crumpet during baking.

3.2.4.1 DSC of emulsifiers

(a) Thermal transitions occurring during heating of emulsifiers were observed using the DSC. Approximately 3 mg emulsifier were accurately weighed into the sample pan, sealed and reweighed as described in Section 3.1.9.1. Samples were heated from 7 to 120°C at 10 C°/min against a reference pan containing a sufficient mass of sand to match as closely as possible the heat capacity of the sample. Samples were reheated in the DSC immediately after the first run and subsequent cooling to study the reversibility of the observed transitions.

(b) Thermal transitions occurring during heating of emulsifiers in the presence of water were also studied.

(i) Emulsifier was weighed into the DSC pan and 10 μl water were added using a 10 μl syringe (Hamilton Co., Reno, NV, USA). The sample mass was 0.2 mg for MG and 0.6 mg for SSL.

(ii) Emulsifier dispersions were made and stored overnight at room temperature prior to DSC analysis of 10 mg samples.

SSL dispersion was made by adding 0.6 g SSL to 10 g water with stirring; MG dispersion was made by adding 0.28 g MG to 14 ml water at 60°C with vigorous stirring until cooled to room temperature.

DSC analysis was carried out as described for the emulsifier powders (Section 3.2.4.1(a)).

3.3 Model Batter Studies

3.3.1 Preparation of the batter

Batter was prepared in the laboratory using a batch weight based on 200 g total flour. The leavening acids, sodium bicarbonate and vinegar were omitted from the formulation for this part of the study since it was expected that CO_2 released would cause the hermetically-sealed DSC pans to burst. This practice has been adopted previously by Abboud and Hoseney (1984).

3.3.2 DSC studies of the batter

3.3.2.1 Trial I

Control batter was prepared as above. Two batches containing emulsifiers were also prepared. MG and SSL were added during mixing in their powder form at levels of 0.14% and 0.30% of flour weight, respectively. These levels were used since they gave batter of good machinability under factory conditions.

Batter samples of about 10 mg were transferred to DSC pans and heated using a heating program designed to imitate the cooking procedure of the central portion of a cooking crumpet as determined by the measurement of the temperature profile (Section 3.2.3). This involved heating from 20 to 85°C at a rate of 20 C°/min, using sand as a reference. "Cooked" samples prepared in this way were used for a short storage trial (Storage Trial I) as described in Section

3.5.1.1.

3.3.2.2 Trial II

Control batter was prepared as in Trial I. Emulsifiers were added at the same levels as in Trial I but as a dispersion. SSL dispersion was made by adding 0.6 g SSL to 10 g water with stirring, and leaving overnight at room temperature. MG dispersion was prepared by adding 0.28 g of MG to 14 ml water at 60°C with vigorous stirring until cooled to room temperature at which it was stored overnight. The dispersions were added to the batter at the mixing stage.

DSC runs were carried out using 10 mg batter samples with sand as the reference. The heating program was from 7 to 125°C at a rate of 10 C°/min. Samples were also "cooked" as for Trial I to be used in Storage Trial II (Section 3.5.1.2).

3.3.2.3 Trial III

Control batter was prepared as in Trial I. Batters were also prepared with higher emulsifier levels than used in previous trials. Both MG and SSL were added at 0.5% flour weight, in powder form as in Trial I and in dispersion form as in Trial II.

DSC runs were carried out using 10 mg samples with sand as the reference and heating from 7 to 125°C at a rate of 10 C°/min.

3.4 Physicochemical Characteristics of Crumpets and Batters

3.4.1 Moisture determination

Moisture content of the crumpets and batters was determined by loss of weight of a 2 g sample after heating in a forced air oven at 110°C for 20 h.

3.4.2 pH determination of crumpets and batters

pH determinations were made using a Fisher Accumet Selective Ion Analyzer Model 750 (Fisher Scientific Co., Pittsburgh, PA, USA) in pH mode.

Batter was used "as is", without further treatment, for pH determination, while for crumpets 10 g of sample were blended with 40 g distilled water prior to pH determination.

3.4.3 Scanning electron microscopy

Samples to be observed in the SEM must be dried to a low moisture content prior to the gold-coating stage. Two sample preparation methods, modified versions of those methods reported by Varriano-Marston (1977) to give excellent results for bread dough samples, were used in the present study.

3.4.3.1 Vacuum desiccation

Cubes, ca. 1 cm^3, of crumpet or batter, ca. 2 ml, were vacuum dried at room temperature for 48 h in a desiccator containing phosphorus pentoxide. Samples were then fractured so that the interior could be observed.

3.4.3.2 Freeze drying

Cubes, ca. 1 cm^3, of crumpet or batter, ca. 0.5 ml, were frozen in isopentane cooled by liquid nitrogen. This freezing method is preferable to freezing directly in liquid nitrogen since the isopentane does not boil around the specimen, which would slow down the freezing rate (Angold, 1979). The frozen samples were then transferred to liquid nitrogen and fractured.

Two freeze-drying methods have been used. The first involved the use of an Edwards Pearce Tissue Dryer (Edwards High Vacuum Canada Ltd., Richmond, BC) containing phosphorus pentoxide to absorb moisture. Samples were dried at -40°C for 36 to 48 h.

The second method used a Model FFD-42-WS Freeze Dryer (The Virtis Co. Inc., Gardiner, NY, USA) with a shelf temperature of -15°C and a drying time of 48 h.

After the drying procedure, specimens were mounted on aluminum stubs using silver paint, coated with 20 nm gold and examined in either a Stereoscan 100 or Stereoscan 250 SEM (Cambridge Instruments Ltd., Cambridge, U.K.) at an accelerating potential of 10 kV. The two freeze-drying methods were found to produce similar samples and were used interchangeably.

3.4.4 Light microscopy

3.4.4.1 Sample preparation

Two methods have been used to prepare samples for sectioning prior to light microscopy, both involving the use of a cryostat (Cryo-Cut Microtome, American Optical Corp., Buffalo, NY, USA) thermostatted at -20°C. The cryostat has advantages over traditional methods where microscopical examination of food is concerned. When moist foods are frozen, the ice formed acts as the support required for sectioning, thus avoiding an embedding procedure. Both fixed and unfixed material can be sectioned (Flint, 1982).

(a) Sample preparation method I

This was a modified version of that described by Moss (1972). Cubes, ca. 1 cm^3, were cut from fresh crumpets using a scalpel and rapidly frozen in isopentane cooled by liquid nitrogen. Frozen samples were attached to the cryostat object disks using a slurry consisting of carboxymethylcellulose and water, covered with plastic film and placed in the cryostat chamber to warm up to -20°C prior to sectioning. This preparation method was found to have a drawback due to the very open nature of the crumpet sample.

(b) Sample preparation method II

In this method, a proprietary formulation of water-soluble glycols and resins (Tissue-Tek II OCT Compound, Miles Lab-Tek Division, Miles Laboratories Inc., Naperville, IL, USA) was used both to embed the sample and to attach the sample to the cryostat object disk.

The sample cube was placed in a small plastic container and covered with OCT compound. The sample was then vacuum infiltrated by placing the container in a desiccator attached to an aspirator. When no more air bubbles were seen to escape (approximately 2-3 h) the vacuum was slowly released. The sample was then frozen with liquid nitrogen and mounted on the cryostat object disk using a few drops of OCT compound, covered with plastic wrap to prevent dehydration and placed in the cryostat chamber to warm to -20°C prior to sectioning.

Samples prepared by either method were sectioned as follows: sections of 10 µm thickness were cut, transferred from the knife to clean glass slides at room temperature onto which they flattened. The sections attached to the slides were allowed to thaw and dry for 24 h prior to staining with the specific reagents.

Unstained samples were observed in polarized light to differentiate birefringent starch granules (Zeiss-Winkel standard polarizing microscope, R. Winkel GMBH, Göttingen, Germany).

3.4.4.2 Staining procedure

Details for the preparation of the reagents are given in Appendix I.

(a) Periodic Acid Schiff (PAS) / Fast Green procedure

Sections dried on the slide were covered with water, then immersed in periodate solution for 5 min at room temperature. After washing in water, sections were immersed in the reducing rinse to remove traces of periodate. Water washing was repeated, after which the slides were covered with Schiff's reagent for 20-30 min at room temperature. Sections were then washed three times with sulphite water followed by distilled water. Sections were counterstained for protein by using Fast Green FCF for 5 min followed by water washing.

Some samples were simply covered by a coverslip prior to observation, thus using water as the mountant. Other samples were dehydrated in an ethanol series followed by 99.5% acetone. The sections were cleared using a series of acetone-xylene mixtures ending with xylene and mounted with DPX mountant (BDH Chemicals, Toronto, Ont.).

After this staining procedure, carbohydrates appeared magenta and proteins were coloured green.

Control samples were treated as above but omitting the periodate rinse.

Lack of magenta stain in the control confirmed that a positive reaction in the other sections was due to carbohydrates.

(b) Protein staining

Some sections were stained only for protein by using the Fast Green FCF procedure as described in Section 3.4.4.2(a).

(c) Iodine staining

Sections were stained by immersion for about 15 min in a working solution of Gram's Iodine.

After staining, sections were observed using an Olympus BH Microscope (Olympus, Tokyo, Japan).

3.5 Storage Trials

3.5.1 Short-term storage

Batter samples, having been "cooked" on the DSC as described in Section 3.3.1, were stored in the DSC pans up to 6 days at 5°C±1 C° and at 25.0°C±0.5 C°. The size of the "staling endotherm" was followed using DSC thermograms obtained on days 0, 1, 3 and 6, by heating from 7 to 125°C at a rate of 10 C°/min. Different masses of sand were used as references. Enthalpy involved in melting the starch crystallites was obtained by measuring the area of the endotherm peak and using the formula given in Equation 3.4. Characteristic temperatures T_o, T_p and T_c were determined as described in Section 3.1.10. The endotherm due to breakdown of the amylose-lipid complex was also studied throughout the storage period.

3.5.1.1 Storage Trial I

Cooked batter samples prepared as described in Section 3.3.2.1, Trial I were used for this storage trial.

3.5.1.2 Storage Trial II

Cooked batter samples prepared as described in Section 3.3.2.2, Trial II were used for Storage Trial II.

3.5.2 Long-term storage

3.5.2.1 Storage Trial III

Crumpets were prepared in the factory as detailed in Section 3.2.1.2. In addition to the control batch, two batches with emulsifiers were manufactured. To one batch MG was added, mixed with a little of the formula water, at a level of 0.14% of flour weight. The third batch contained SSL at a level of 0.3% of flour weight, also added mixed with some of the formula water. On arrival at the laboratory the packaged crumpets to be used in the storage trial were put into one of two rooms. One storage room was held at 5°C±1 C°, the other at 25°C±0.5 C°.

Samples were assessed for changes using the techniques described in Section 3.5.3. Samples stored at 25°C were tested on days 1, 4, 7, 15, 22, 37, 43 and 60, while samples stored at 5°C were tested on days 1, 3, 6, 10, 16, 21, 34, 44 and 59. It was not feasible to test samples from both storage temperatures on the same day due to lack of manpower and instrumental capabilities.

3.5.2.2 Storage Trial IV

For this storage trial three batches of crumpets, being a control batch and two batches containing different levels of SSL, were manufactured in the factory. SSL was prepared for addition to the batch by adding the required mass with stirring to some of the formula water to a level of 6% (w/w). The dispersion was left at room temperature for about 20 h after which it was a translucent, viscous fluid, then added to the batch after the flours. The two levels of SSL used were 0.3% and 0.45% of flour weight.

On arrival at the laboratory, packaged crumpets were stored at 5°C and 25°C. Those stored at 5°C were assessed for changes during storage on days 1, 4, 7, 10, 14 and 21; those stored at 25°C were tested on days 1, 3, 6, 9, 15 and 22.

3.5.3 Techniques to follow changes during storage

3.5.3.1 Compression methods

Compression of samples to assess their firmness was carried out using an Instron Universal Testing Instrument Model 1132 (Instron Corp., Canton, MA, USA). Changes in firmness of samples were measured during both Storage Trials III and IV.

Two compression methods were used.

Crumpet compression

Measurements were made on the four crumpets in the centre of the pack. A square prism was removed from the central area of each crumpet, as shown in Figure 3.3, and wrapped in aluminum foil to minimize dehydration prior to

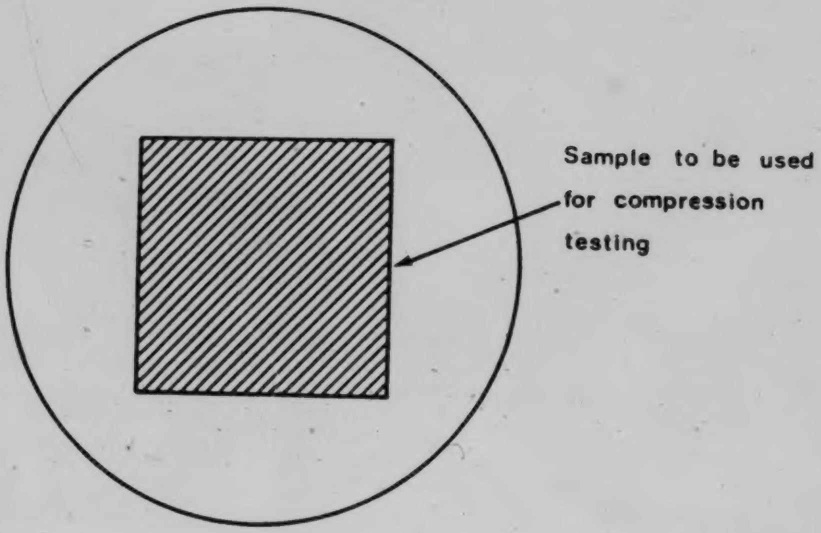

Sample to be used for compression testing

Figure 3.3 Position of sample from crumpet used for compression testing.

analysis. Each of the four replicates was compressed using a 3.55 cm diameter compression anvil to 33% of the original height. Crosshead speed was 5 cm/min and chart speed was 20 cm/min.

A typical Instron output for crumpet compression is shown in Figure 3.4. Firmness values were obtained from the initial section of this force-deformation curve as carried out by Guy (1983). To balance the slight deviation of the crumpet slices from parallelity, the linear part of the curve was extrapolated to the axis to obtain a point interpreted as the beginning of compression (Mälkki *et al.*, 1978) as shown in Figure 3.4. The slope of this section of the curve in kgf/mm was used as the firmness value.

Cell wall material compression

A modified version of the method described by Guy and Wren (1968) was used to follow changes in firmness of the crumpet "cell wall material".

On receipt of crumpet samples at the laboratory from the factory the required number of packages were removed from the consignment prior to storage. Crumpets were removed from the packages and the lower crust was sliced off and discarded. The remaining part was cut into smaller pieces and "blended" in an Osterizer Galaxie 8 (Sunbeam Corp. [Canada] Ltd.) using the "grate" setting for 7 s. Portions of 10.0 g of the "blended" crumpet were weighed into 50 ml centrifuge tubes, sealed with Parafilm (American Can Co.,

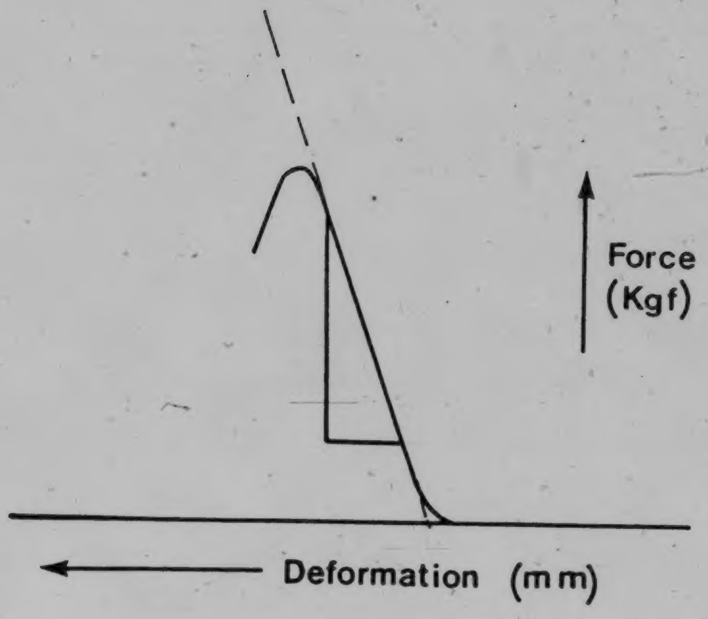


Figure 3.4 Typical Instron curve from crumpet compression.

Greenwich, CT, USA) and centrifuged at 2,000 rpm (850 g) for 15 min using a centrifuge fitted with a swinging bucket rotor (Model S2K, International Equipment Co., Needham Hts., MA, USA) in order to pack the crumb in the bottom of the tube. The Parafilm was removed and the tubes were packed, three to a pouch. Packaging was accomplished using a Multivac AG500 vacuum/gas packager (W.R. Grace & Co., Ajax, Ont.) delivering a gas mixture to match that used at the factory. The proportion of gas from separate cylinders was regulated by a Smith's Proportional Mixer Model No. 299-006-1 (Tescom, Minneapolis, MN, USA). The atmosphere in the packages was checked using the GC technique previously described (Section 3.2.2). The packaged tubes were then put in storage at 5 and 25°C prior to compression testing.

Firmness of the cell-wall material was determined by compressing the sample with a 12.5 mm diameter probe for a distance of 1 cm. Crosshead speed was 2 cm/min and chart speed was 20 cm/min. A typical force-deformation curve obtained by this method is shown in Figure 3.5. Firmness in kgf/mm was obtained from the gradient of the initial linear portion of the curve as described in Section 3.5.3.1(a).

3.5.3.2 DSC measurement

The staling endotherm and the amylose-lipid complex endotherm were both followed during Storage Trials III and IV. Samples for DSC analysis were obtained from the inner region of the crumpet after compression testing. Pieces of crumb of about 10 mg were removed from two crumpets and

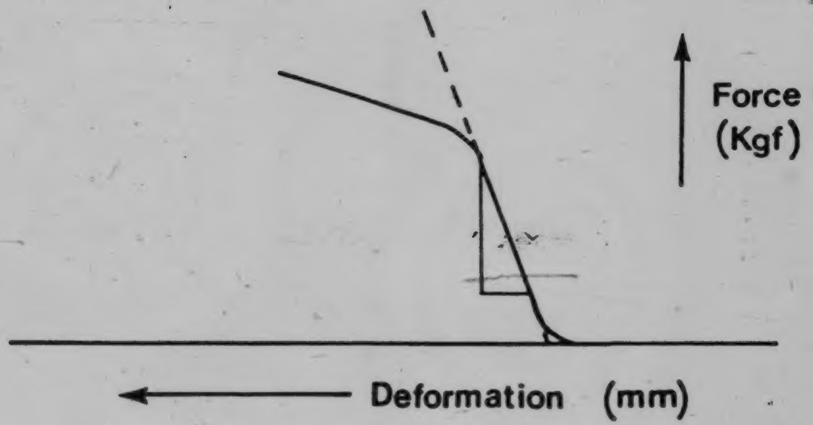


Figure 3.5 Typical Instron curve from cell-wall material compression.

three, four or five replicates were run. Scans were carried out between 7 and 125°C heating at a rate of 10 C°/min.

3.5.3.3 X-ray diffraction

Samples from Storage Trial III only were subjected to X-ray diffraction analysis on the following test days: 1, 10, 16 and 30. Preliminary experiments comparing the two sample preparation methods described by Dragsdorf and Varriano-Marston (1980) revealed that X-ray diffraction of starch washed out from crumpets resulted in a reduced amorphous background compared to that obtained in the X-ray diffraction pattern of crumpet crumb. However, since it was also found that the starch peaks became somewhat less prominent and bearing in mind the possibility of introducing artefacts during the former preparation method and the ease of preparation for the latter method, all X-ray analysis was performed on compressed crumpet crumb.

Sections of the crumpet interior were removed using a scalpel and pressed into an aluminum sample holder using a glass slide to obtain a surface that was as smooth as possible. X-ray patterns were obtained by using a Philips X-ray diffractometer with a vertical goniometer. The intrument used Co Kα radiation modulated by a graphite monochromator and was fitted with a xenon gas flow proportional counter detector. Operation was at 50 kV and 20 mA with relative humidity of the sample chamber being kept high (ca. 80%). Samples were scanned from $2\theta = 2°$ to $2\theta = 45°$ at a scan rate of 1° 2θ/min. X-ray patterns were designated according to the d-spacings in Å using Equation 3.5.

$$n\lambda = 2d \sin \theta \qquad [\text{Eq } 3.5]$$

where:

$n = 1$

λ = wavelength of X-rays used (1.7889 Å)

d = interplanar spacings (Å)

θ = angle of incidence of X-rays

Quantification of peaks was achieved by measurement of the area above the extrapolated base line by use of a planimeter.

3.5.4 Avrami analysis of storage data

Data obtained from DSC measurements of the staling endotherm and from the compression testing were fitted to the Avrami equation (Equation 2.1).

3.5.4.1 Application to DSC data

It was assumed that the heats of transition obtained for the staling endotherm were linearly proportional to the mass of crystallized material and that they could therefore be used in the Avrami analysis. Thus:

$$\theta = (\Delta H_L - \Delta H_t)/(\Delta H_L - \Delta H_0) = \exp(-kt^n) \qquad [\text{Eq } 3.6]$$

where:

θ = fraction of crystallizing material not yet crystallized

ΔH_0 = heat of transition at zero time (J/g)

ΔH_t = heat of transition at time, t (J/g)

ΔH_L = heat of transition at infinity (J/g)

k = rate constant (days^{-n})

t = time (days)

n = Avrami exponent

The Avrami exponent is a combined function of the number of dimensions in which crystal growth takes place and the order of the time dependence of the nucleation process. Table 3.5 shows the values for the Avrami exponent for various types of nucleation and growth.

It was assumed that immediately after baking there was no starch crystallization, i.e. $\Delta H_0 = 0$. Equation 3.6 can therefore be rewritten:

$$\Delta H_t = \Delta H_L[1 - \exp(-kt^n)] \qquad \text{[Eq 3.7]}$$

The three unknown parameters, ΔH_L, k and n, were estimated simultaneously by an iterative non-linear weighted least squares procedure (BMDPAR; BMDP, 1983).

In non-weighted regression analysis there is an assumption of homogeneous variance. When data are used where the observations have unequal variances, this assumption is not justified and weighted analysis must be used; the weights depending upon the precision of the observations and being the reciprocals of the variances (Steel and Torrie, 1980). To obtain the best fit, the weighted residual sum of squares is minimized.

3.5.4.2 Application to compression data

In applying the Avrami analysis to data derived from compression tests it is assumed that increasing firmness of the sample is due to an increasing degree of crystallization

Table 3.5 Values for Avrami exponent, n, for various types of nucleation and growth.

n	Nucleation and growth
3+1 = 4	Spherulitic growth (3D), sporadic nuclei
3+0 = 3	Spherulitic growth (3D), instantaneous nuclei
2+1 = 3	Disk-like growth (2D), sporadic nuclei
2+0 = 2	Disk-like growth (2D), instantaneous nuclei
1+1 = 2	Rod-like growth (1D), sporadic nuclei
1+0 = 1	Rod-like growth (1D), instantaneous nuclei

of the starch fraction. Thus, the relevant Avrami equation is as follows:

$$\theta = (F_\infty - F_t)/(F_\infty - F_0) = \exp(-kt^n) \qquad \text{[Eq 3.8]}$$

where:

F_0 = firmness at zero time (kgf/mm)

F_t = firmness at time t (kgf/mm)

F_∞ = firmness at infinity (kgf/mm)

θ, k, t, n are as previously defined (Eq. 3.6).

This case differs from the Avrami equation for DSC data in that F_0 is not equal to zero. For the analysis F_0 was given the value of the average of the initial firmness measurements, determined approximately 18 h after manufacture. Equation 3.8 may therefore be rewritten:

$$F_t = F_\infty - \{[F_\infty - F_0][\exp(-kt^n)]\} \qquad \text{[Eq 3.9]}$$

The three unknown parameters, F_∞, k and n, were estimated simultaneously for the DSC data (Section 3.5.4.1).

The usual tests which are carried out to assess a linear model are generally not appropriate when the model used is nonlinear (Draper and Smith, 1981). However, test statistics can be calculated to give an indication of the fit and appropriateness of the model. The square of the multiple correlation coefficient, R^2, was calculated from the weighted sums of squares as:

$$R^2 = (SSY - SSE)/SSY \qquad \text{[Eq 3.10]}$$

where:

SSY = total sum of squares, weighted

SSE = residual sum of squares, weighted

R^2 gives an indication of the amount of variation of data that is explained by the model but does not give an indication of the appropriateness of the model. In the linear model case, the F-statistic for lack of fit is used to assess appropriateness of the model. In the nonlinear case a similar statistic (here denoted "F") was calculated in order to give some indication of model appropriateness (Draper and Smith, 1981). The residual sum of squares (SSE) is made up of two parts, namely the sum of squares for pure error (SS_{pe}) resulting from variation of replicates about the sample mean at each sampling point and the sum of squares for lack of fit (SS_{lof}) which is a result of the deviation of sample means from the value predicted by the model for each sampling point. All sums of squares are weighted. The "F"-statistic was calculated using equation 3.11:

$$\text{"F"} = MS_{lof} / MS_{pe} \qquad \text{[Eq 3.11]}$$

where:

MS_{lof} = mean square for lack of fit

MS_{pe} = mean square for pure error

The value obtained was compared against the table value with the appropriate degrees of freedom. Confidence intervals for the Avrami parameters were calculated using their standard errors obtained from the output of the computer program.

4. RESULTS AND DISCUSSION

4.1 Physicochemical Characteristics of Flours and Starches

The moisture contents of the flours and starches used in this study are shown in Table 4.1. These properties were required to enable other quantities determined to be quoted on a dry basis.

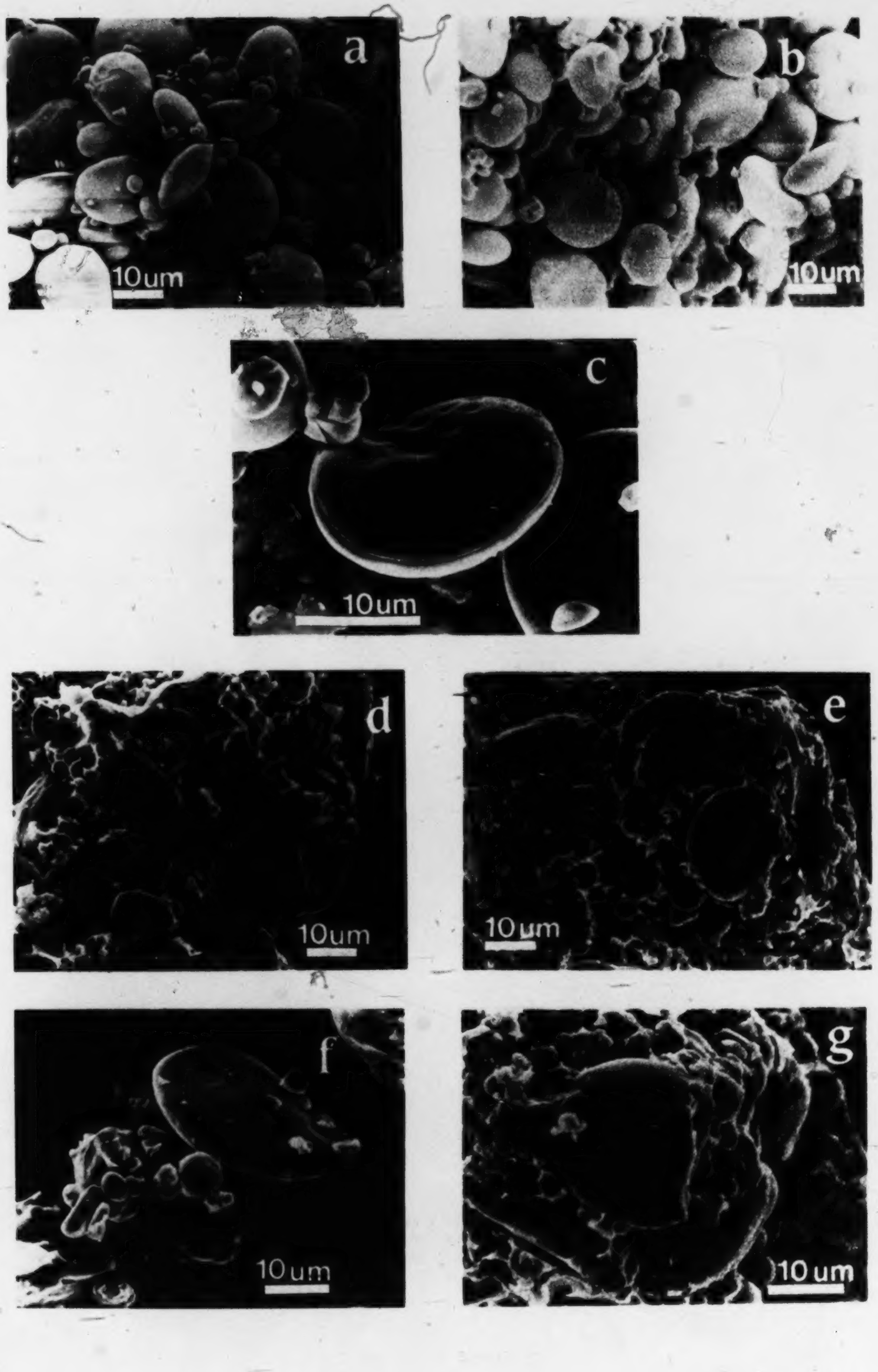
Scanning electron micrographs of the isolated starches and flours are shown in Plate 4.1. The typical bimodal distribution of the wheat starch granule is clearly demonstrated in Plate 4.1 (a and b) and the equatorial groove of the large, lenticular A-granule can be seen in Plate 4.1 (c). Both samples showed a small amount of protein contamination. In the flour particle, the majority of the starch granules are held together in a matrix. There was a tendency for a greater number of starch granules, principally the small B-granules, to be free in the Primrose flour (4.1. d and f) than in the Five Roses flour (4.1. e and g). It is well known that flour milled from hard wheat contains more damaged starch than flour milled from soft wheat (MacRitchie, 1980). This arises as a consequence of the coherent nature of the endosperm. After the initial tendency for splitting to occur along the plane of the cell walls, increasing pressure causes fracturing across the cell. Starch granules are very firmly embedded in the matrix, so this cleavage can result in physical damage to a proportion of the starch granules. In soft wheats starch

Table 4.1 Moisture contents of flours and starches.

Sample	Moisture content[1] (% wb)
Five Roses flour	11.29
Five Roses starch	13.02
Primrose flour	9.60
Primrose starch	12.15

[1] Mean of triplicate determinations.

Plate 4.1. SE-Micrographs of starches and flours. (a) Primrose starch; (b),(c) Five Roses starch; (d) Primrose flour; (e) Five Roses flour; (f) Primrose flour; (g) Five Roses flour.


a
10um
b
10um
c
10um
d
10um
e
10um
f
10um
g
10 um

granules are held together less tightly so that pressure during milling causes them to break away more easily, therefore suffering less damage. It was not possible to quantify starch damage levels in the flour and starch samples by observation with the SEM so chemical means were used. Table 4.2 shows the levels of damaged starch in both flour and isolated starch samples. Values are given in Absorbance units x 10 and also converted to % damage (Farrand, 1964) using the regression equation calculated by Williams and Fegol (1969). These latter researchers assessed 53 flours, representing a wide range of starch damage, wheat variety and environment for starch damage and found levels of between ca. 1 (5.3%) and 11 units (55.6%). Thus it can be seen that the starch damage level in Five Roses flour is extremely high compared to that in Primrose flour (14 units vs. 3 units). This result was expected since the former flour is milled from hard wheat while the latter is from soft wheat. The results for the two starch samples shown in Table 4.2 reveal that, since only prime starch was recovered with much of the damaged starch being discarded during the fractionation process, the amount of damage was low (0.5 units for Five Roses starch; 0.3 units for Primrose starch).

The protein content (nitrogen x 5.7) of both starch isolates was determined to be at a level of 0.3%, indicating good separation.

Swelling and solubility patterns of starches isolated from Five Roses and Primrose flours are shown in Figures 4.1

Table 4.2 Level of starch damage in flour and starches used.

Sample	Starch damage[1] (Absorbance x 10)	(%)[2]
Five Roses flour	14.0	70.7
Five Roses starch	0.5	2.8
Primrose flour	3.0	15.4
Primrose starch	0.3	1.8

[1] Mean of duplicate determinations.

[2] Obtained using $y = 0.286 + 50.3x$ where y is in %; x is in Absorbance units.

and 4.2, respectively. Means and standard deviations are presented in Appendix II, Tables 8.1 and 8.2. Such patterns have been used previously to study the associative bonding in starch granules and the influence of such factors as surfactants on starch gelatinization properties (Collison, 1968). It can be noted in Figure 4.1 that starch isolated from Five Roses flour has a consistently lower swelling power over the whole range of the experiment. A possible implication of this is that bonding in the starch granules of Five Roses flour is stronger than in those of Primrose flour, thus allowing less swelling to take place. Another plausible explanation could be that a higher damage level in Primrose starch facilitated greater swelling than in Five Roses starch. However, since results showed that damage levels were equally low, this argument is nullified.

Ghiasi *et al.* (1982a) carried out swelling power and solubility experiments on prime starch isolated from experimentally milled hard wheat flour. Their results showed a two-step swelling pattern which tended to be smoothed by addition of surfactants. Kulp (1973) studied swelling power and solubility of starches isolated from commercially milled hard red spring, hard red winter and soft red winter wheat flours which had been defatted. Swelling power of the hard red spring and soft red winter starches were very similar over the temperature range from 60 to 90°C but at higher temperatures the latter starch tended to swell more.

Ghiasi *et al.* (1982a) found that solubility of starch

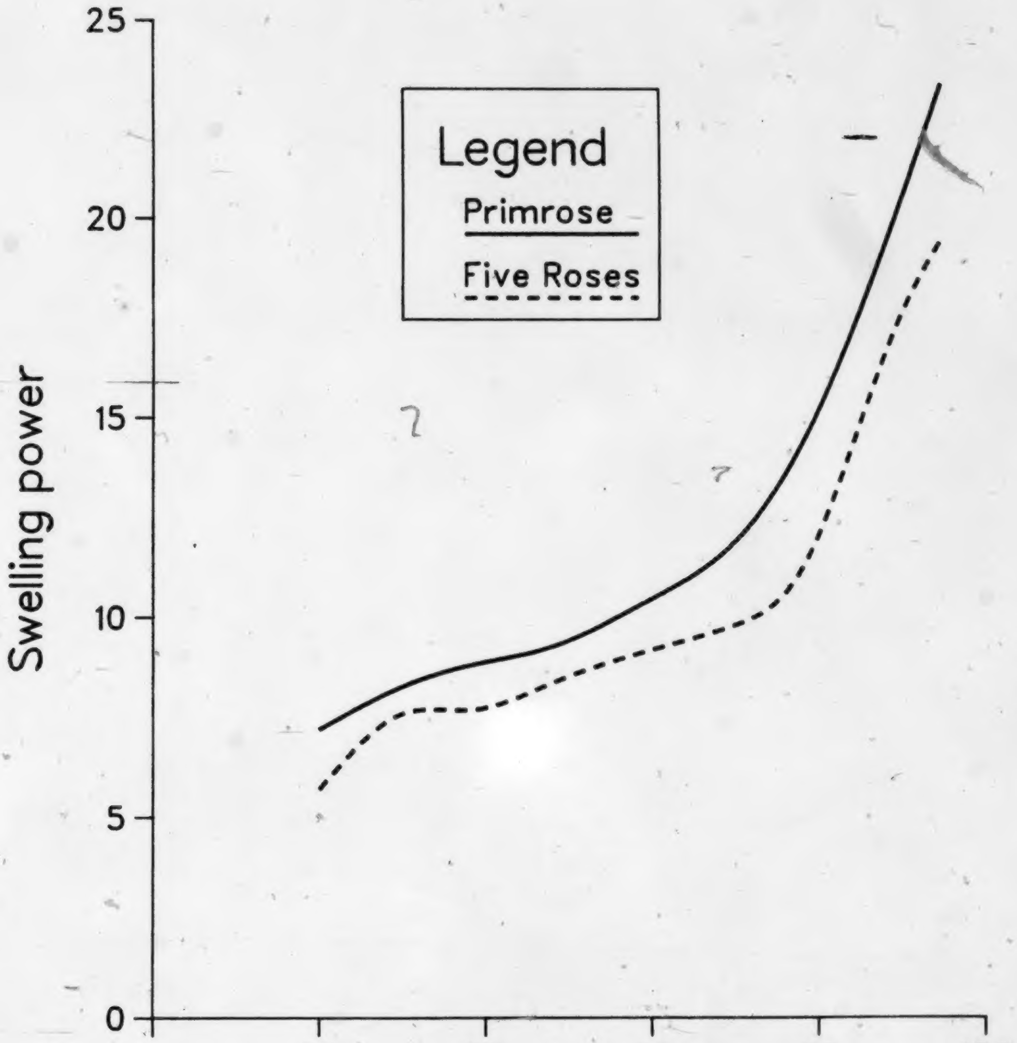


Figure 4.1 Swelling power of the starches as a function of temperature.

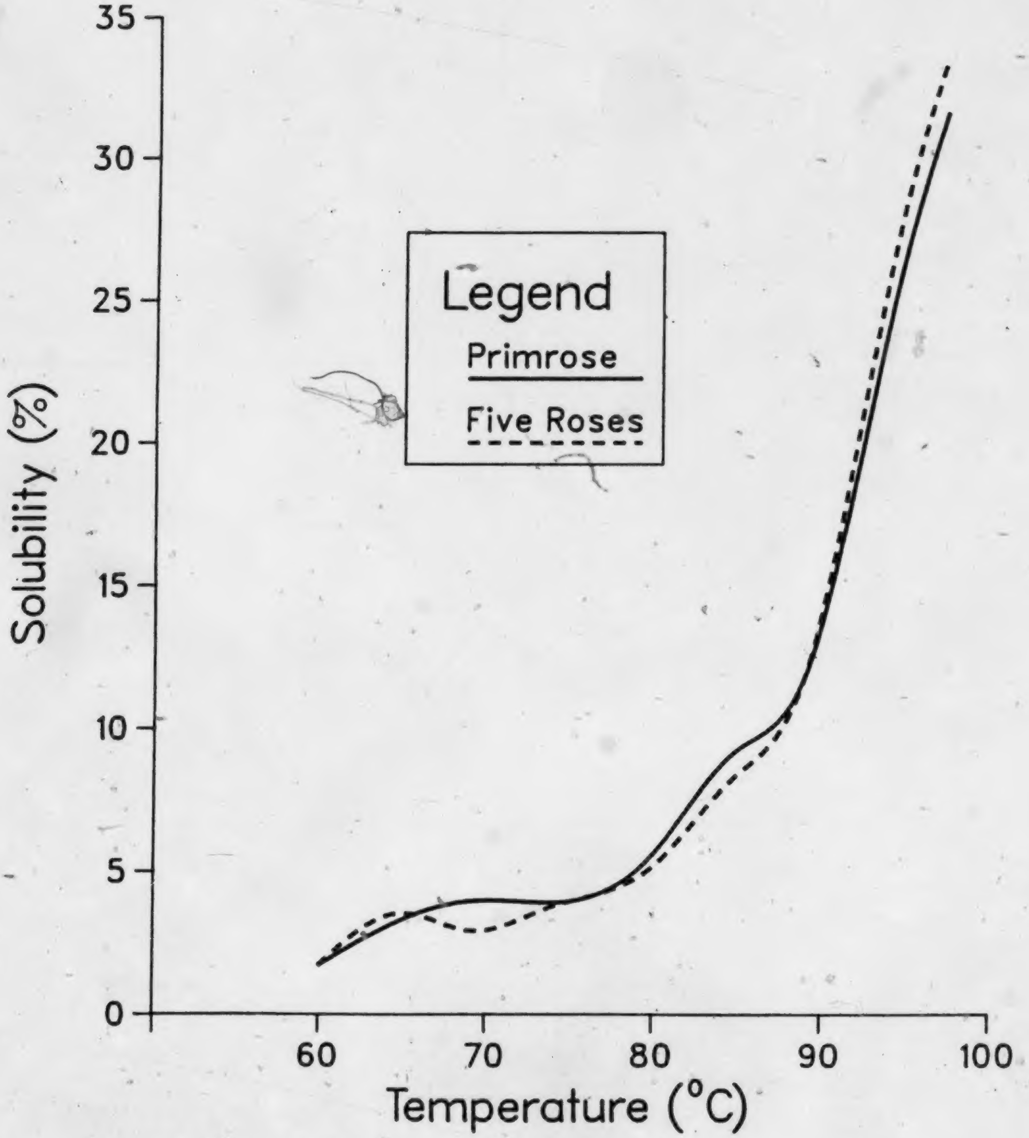


Figure 4.2 Solubility of the starches as a function of temperature.

paralleled swelling power for their control starch and for the surfactant-treated sample. Figure 4.2 shows that solubility of Five Roses starch is essentially equivalent to that of Primrose starch at any given temperature. Kulp (1973) found that solubilities of hard red spring and soft red winter starches were similar up to around 80°C, above which the soft flour was more highly solubilized, the difference being very marked at 95°C. In this respect, the results of Kulp (1973) agree with those of Ghiasi *et al.* (1982a) in that a higher swelling power is paralleled by a higher solubility. In the present study, the different swelling powers exhibited by the two starches were not matched with differing solubilities. In fact, the solubilities of Primrose and Five Roses starches were similar over most of the temperature range studied, with small differences being noted at 70 and 85°C.

The solubles leached from the granules during the solubility and swelling power characterization were analyzed for amylose content. Results in terms of mg amylose leached into a 25 ml aliquot of supernatant as a function of temperature are presented in Figure 4.3. Means and standard deviations are given in Appendix II, Table 7.3. It can be seen that the graph follows the same general form of the solubility curve.

When amylose is presented as a percentage of solubles leached from the starch, Figure 4.4, it is very difficult to make any conclusions regarding trends and differences

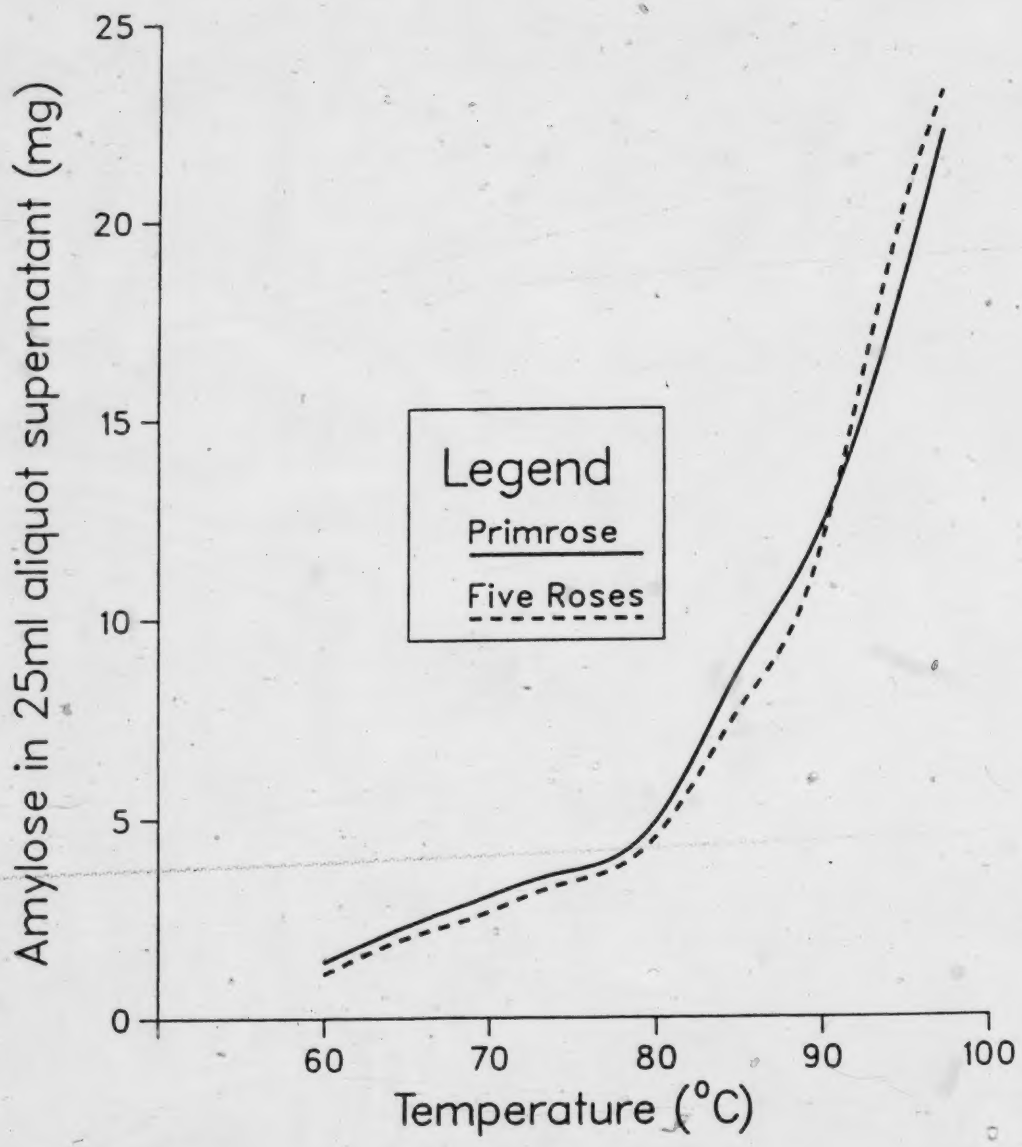


Figure 4.3 Amylose solubility of the starches (mass of amylose leached into supernatant).

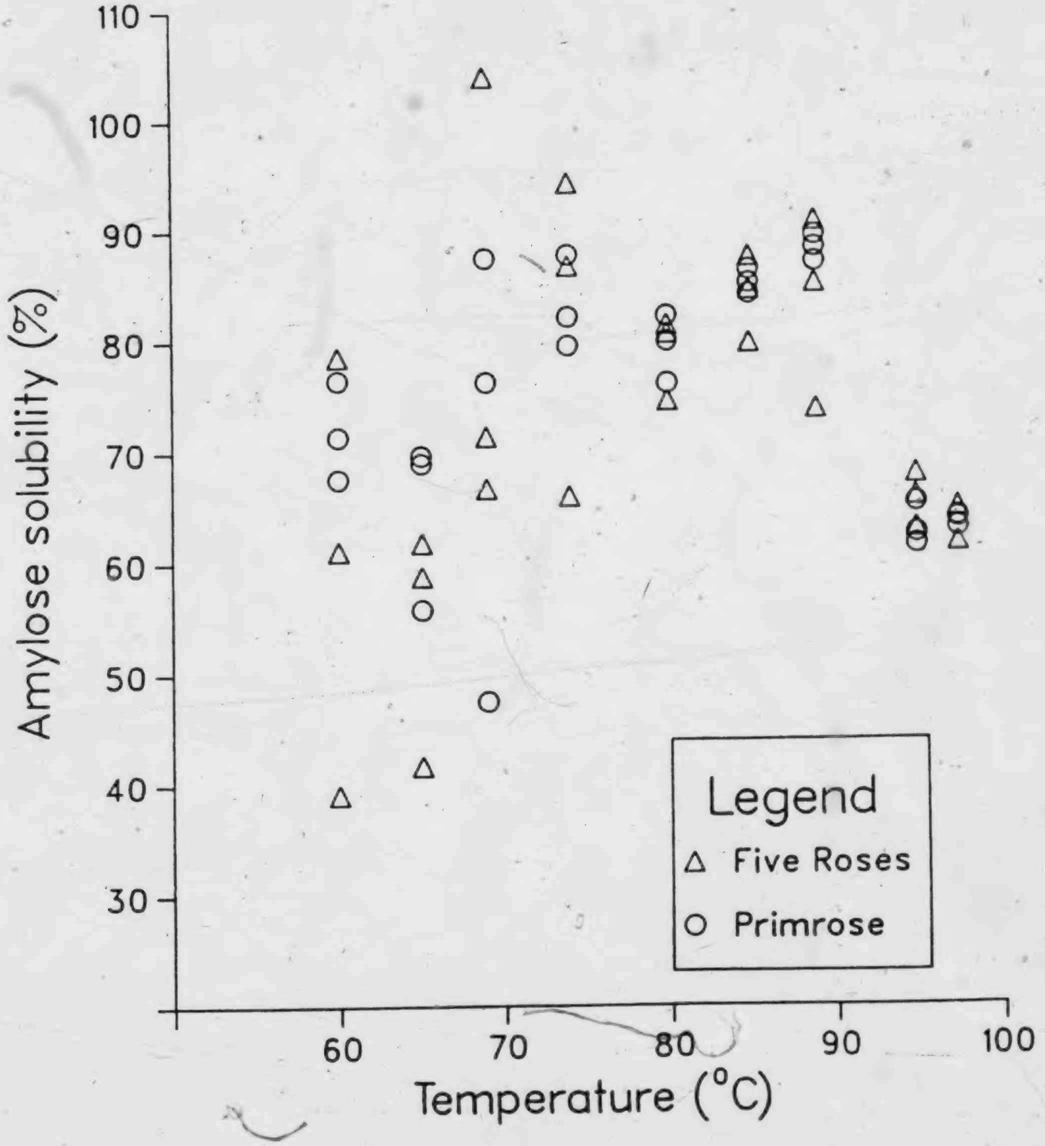


Figure 4.4 Amylose solubility of the starches (% amylose in solubles).

between the two starches due to the marked variation which is especially large at temperatures below 80°C. This temperature corresponds to the beginning of the second step in both the solubility curve and the amylose curve below which masses of solubles are extremely small.

Ghiasi *et al.* (1982a) found that iodine affinity (measured using the method of Schoch [1964b]) of leached solubles decreased from 75 to 120°C for the control, while that of surfactant-treated starch increased to 95°C and then decreased to 120°C. They found, therefore, that at lower pasting temperatures amylose was preferentially leached from control granules, while amylopectin was gradually solubilized at higher temperatures. Ghiasi *et al.* (1982a) concluded that, even though iodine affinities of the treated granules were low below 95°C, it was impossible to state that at these temperatures amylopectin was preferentially leached due to the very low level of extractable solubles. In a similar study on a commercial wheat starch (Hill and Dronzek, 1973) it was found that solubility of starch increased rapidly from 55 to 65°C and amylose, expressed as a percentage of the solubilized starch, paralleled total solubility. The implication of this is that, at least up to about 65°C, iodine affinity of total solubles increased markedly and that from 55 to about 61°C amylopectin was preferentially leached, since in that temperature range amylose content was less than 50%. Hill and Dronzek (1973) gave no data for percentage of soluble solids present as amylose at temperatures higher than 65°C; however, at that temperature, the figure of 90% had been reached and the trend was sharply upwards. These results are somewhat anomalous; it is generally understood that since amylose is a smaller molecule than amylopectin (MW amylose ≃ 140,000; MW amylopectin ≃ 4,000,000 (D'Appolonia *et al*., 1971)) it should be leached preferentially (Greenwood, 1976). In the present study, the percentage of leached solubles being amylose was, in all but three determinations, above 50% over the whole experimental range, as shown in Figure 4.4. A slight upward trend was seen between 80 and 90°C where amylose constituted about 85 to 90% of solubles, after which there was a decrease to about 65% at 97.5°C. Both starches followed the same pattern.

Viscosity of dilute suspensions of starches and flours was studied; the results are presented graphically in Figure 4.5. As is usual with the starch-water system (Greup and Hintzer, 1953), samples exhibited thixotropy (time dependent, shear thinning). Results shown in Figure 4.5 indicate that Primrose starch has a higher apparent viscosity than Five Roses starch, thus paralleling the swelling power data. Apparent viscosities of both Primrose samples are higher than those of Five Roses samples over the whole range of shear rates used in the study.

The increase in viscosity of a starch suspension on gelatinization has been attributed to the granules imbibing increasing amounts of free water as they swell, thus making

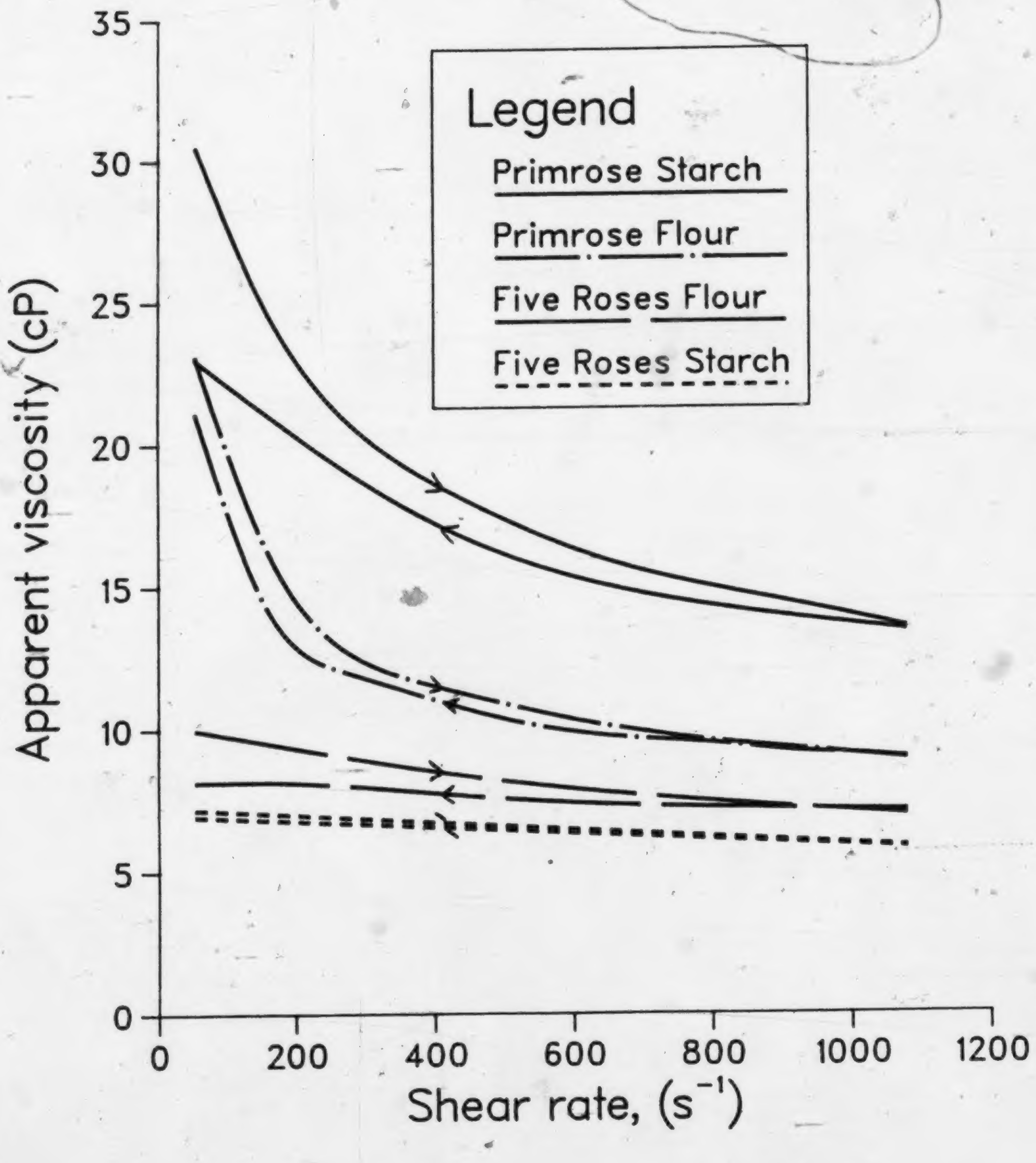


Figure 4.5 Viscosity of starches and flours.

contact between them more likely (Collison, 1968; Schoch, 1965). Miller *et al.* (1973) found that granule swelling caused the initial viscosity increase but did not account for the rapid rise in viscosity of a wheat starch suspension heated in excess water. They concluded that exudation of a filamentous network from the starch granules was principally responsible. Evans and Haisman (1979) made studies of gelatinized starch suspensions under well-defined flow characteristics using a rotary viscometer. In addition to studies of commercial starch preparations they ran tests on wheat flour which had been heat treated to inactivate naturally-occurring enzymes. In steady shear, wheat flour showed very similar behaviour to the starches, while in oscillatory shear it differed markedly. The explanation proposed was that in addition to swollen granules there was an interconnecting network, presumably composed mainly of protein. Since the heat-treated wheat flour behaved like the granular starches in steady shear it seemed that the network was broken down during continuous shearing.

Solubilities of Primrose and Five Roses starches were essentially equal over the tested temperature range while Primrose starch had a somewhat higher swelling power than Five Roses starch. This suggests that, while the exudate may be responsible for the viscosity increase on heating, it is the difference in swelling power that causes the viscosity of the Primrose starch suspension to be greater than that of Five Roses starch.

Figure 4.5 shows that the apparent viscosity of Primrose starch was higher than that of Primrose flour over the entire range of shear rates used. The Five Roses flour and starch suspensions did not behave in the same manner; apparent viscosity of the flour suspension was very slightly greater than that of the starch. There are several differences between the two flours that may account for the different responses to the experiments described above. Primrose flour is milled from a soft wheat, is chlorinated and contains a damaged starch level of 3 units. Five Roses flour is from hard wheat with a higher level of damaged starch (14 units) and is not chlorinated.

Cake flour is chlorinated for use in high-ratio cakes (i.e. those containing a higher ratio of sugar and water to flour than traditional cakes) to prevent collapse of the structure. During chlorination of flour, the chlorine reacts with many flour components (Gough *et al.*, 1978). Sollars (1958) concluded that the fundamental difference between chlorinated and untreated flour with regard to cake baking was due to changes in the starch component. However, Huang *et al.* (1982a) used X-ray microanalysis to show that protein absorbed significantly more chloride than did starch during flour chlorination. They also found that chloride uptake by the protein increased with increased chlorine doses while uptake by starch reached a plateau which coincided with the level normally used in commercial practice -and thus concluded that the beneficial effect of chlorination on cake flour is due, at least in part, to the starch component. Chamberlain (1962) proposed that the action of chlorine was to promote granule swelling, but Gough *et al.* (1978) concluded that evidence for this phenomenon was conflicting. Miller *et al.* (1973) studied a dilute gelatinizing starch suspension and found that chlorine suppressed granule folding and inhibited exudate release, thus inhibiting amylograph viscosity development.

Recently, Telloke (1985) studied the effect on starch gelatinization of chlorination of cake flour. He used various concentrations of flour in sucrose solutions of 30-60% w/w to model high-ratio cake batters. Telloke (1985) found that the amount of "amylose" (his notation for linear polymers of glucose long enough to give a blue colour with iodine but not necessarily native amylose as exists within the starch granule) exuded from starch granules during heating and paste viscosity both increased with chlorination of flour, in disagreement with the results of Miller *et al.* (1973). He also noted that initial value and rate of hot paste viscosity rise with increasing chlorination levels varied between the different flours tested thus emphasizing the importance of varietal effect.

Huang *et al.* (1982b) studied swelling power and solubility of a 1% starch suspension using the same method as in the present study (Schoch, 1964a); the starch having been isolated by air classification so as not to affect material adhering to the granule surface which may be removed by wet fractionation (Gough *et al.*, 1978). They observed that swelling power increased as chlorination level increased. Solubility was affected by chlorination but there appeared to be an interaction with the temperature effect. Huang *et al.* (1982a) found that increased levels of chlorine caused a decrease in intrinsic viscosity and an increase in β amylolysis, indicating depolymerization of starch during chlorination. Gough *et al.* (1978) postulated that reaction of chlorine with minor components, chiefly lipids and proteins, associated with the starch granule promotes the changes in starch behaviour. In addition, Gough *et al.* (1978) considered that their location at the granule surface and their alignment could be very important. Thus, the increased swelling power of Primrose starch over Five Roses starch may be due to the effect of chlorination, which could also result in the increased viscosity. However, the starch isolation process involved treatment w[illegible]thyl ether which would have removed much of the lipid material adhering to the starch granule surface. It may be postulated then, that enough of the trace components remained on the granule surface after isolation to exert some effect on gelatinization behaviour.

Recently, Ngo *et al.* (1985) measured the rheological properties of heated cake batters, flour-water and starch-water suspensions prepared using chlorine-treated and untreated cake flours. They found different rheological properties, especially in the temperature range from 90 to

100°C, between the two batters which were not exhibited by the flour and starch suspensions. It was proposed that the beneficial effect of flour chlorination on cake baking was brought about by an interaction of the flour with at least one of the other formula ingredients. Results show that the Primrose starch isolate had a higher apparent viscosity than the flour, which was not the case for the Five Roses samples. It may be that the non-starch components in Primrose flour inhibited starch granule swelling.

Hester *et al*. (1956) concluded that the protein content of flours offered the most obvious explanation for the different gelatinization behaviour of flours and starches, but that the possible role of other constituents of flour should not be overlooked. Takeuchi (1969) determined that a starch-protein interaction, due to attraction of opposite charges, occurred during gelatinization. Olkku and Rha (1978) concluded that protein appears to form complexes with starch molecules on the granule surface, preventing escape of exudate from the granule and therefore interfering with the increase in consistency. They also stated that the role of the protein depends on its state before heat is applied. It is feasible then, that the different viscosities of Primrose starch and flour result from protein-starch interaction. At alkaline pH both starch and protein bear negative charges and complexing does not occur, while at acidic pH protein bears a positive charge and complexes can be formed (Dahle, 1971). Since Primrose flour is chlorinated to an acidic pH (4.60-4.80), more starch-protein complexation may be expected than with Five Roses flour.

Wheat flour contains a very small proportion of both water-soluble and water-insoluble pentosans. It has been estimated that pentosans absorb about a third of the total water in a normal dough (Olkku and Rha, 1978). Therefore it may be possible that this flour constituent could interfere with the water absorption of starches during gelatinization and thus be responsible for some of the differences observed between flours and their isolated starches. The Five Roses flour was not chlorinated and thus its gluten could be developed. The fact that the apparent viscosity of Five Roses flour was slightly greater than that of the corresponding starch may be due to the presence of a protein network of the kind suggested by Evans and Haisman (1979) and referred to previously.

It is known that metal cations have an effect on the gelatinization behaviour of starch granules. Gough and Pybus (1973) established that gelatinization could take place in three distinct forms; type I involved tangential swelling with simultaneous loss of birefringence from hilum to periphery, type II involved both swelling and loss of birefringence from periphery to hilum, and type III involved apparent dispersion of the granule. There were further subdivisions within each type. Gough and Pybus (1973) found that solutions of certain metal chlorides exhibited all three types of gelatinization pattern as the salt concentration increased. Table 4.3 shows mineral contents of the two flours and their isolated starches. Five Roses starch is higher in P, Mg and K, but lower in Ca. It is possible that the different swelling powers of the two starches result from differing mineral content.

Mutual repulsion of ionized phosphate groups tends to enhance swelling of potato starch granules due to ease of entry of water. Neutralization by divalent cations effects reduced swelling and solubility of starch due to the formation of cross-linkages between two phosphoric ester groups either in the same glucose chain or between two adjacent chains (Haydar *et al.*, 1980). A study of the mineral contents of starches and flours in the present experiment showed that all of the phosphate groups were expected to be neutralized. Thus, mutual repulsion of phosphate groups is not a likely explanation for the increased swelling power of Primrose starch. In fact, the higher level of divalent cations present in Primrose starch would be expected to lead to an increased amount of cross-linkages, thus resulting in a decreased swelling power. Since the opposite was found, it was concluded that results obtained for potato starch by Haydar *et al.* (1980) cannot be directly applied to wheat starch under the conditions pertaining to the present study.

When wheat starch is heated in the presence of limited amounts of water, three thermal transitions are observed by calorimetric measurements (Donovan, 1979; Eliasson, 1980).

Table 4.3 Mineral content of starches and flours.

Sample	Mineral content[1] (ppm)[2] P	Ca	Mg	Na	K
Five Roses flour	990.1	103.0	244.6	---[3]	737.0
Primrose flour	806.9	199.4	189.5	---	994.8
Five Roses starch	502.9	57.1	38.9	---	305.5
Primrose starch	425.4	92.9	28.9	---	200.0

[1] Mean of duplicate determinations.
[2] On wet basis.
[3] Lower than detection limit.

At ca. 60°C an endotherm due to gelatinization is observed while a second endotherm at higher temperature may occur when the water content is less than that necessary for unrestricted gelatinization. The third endotherm is observed at temperatures above 100°C and this transition has been interpreted as being due to the presence of an amylose-lipid complex.

It has been shown that heating rate and moisture level have an effect on temperature and heat of gelatinization (Wootton and Bamunuarachchi, 1979) so it is important that these factors be controlled during DSC analysis in order to facilitate comparison between samples. Stevens and Elton (1971) were the first researchers to apply DSC to the study of starch gelatinization and they defined the notation T_o, T_p and T_c to represent the temperatures of gelatinization onset, peak and completion, respectively.

The DSC endotherms obtained for the two flours and their isolated starches are shown in Figure 4.6 (for convenience, all DSC endotherms are shown with a temperature scale on the x-axis although the actual scans were carried out using the time base facility). The corresponding characteristic temperatures and heats of gelatinization (ΔHg) are given in Table 4.4. Of the three temperatures, T_o, T_p and T_c, T_p showed the least variation between replicates of a sample (S.D. = 0.3-0.5). Results for the two starches are equivalent, while the gelatinization endotherms for the flour samples are at slightly elevated temperatures. ΔHg for

Table 4.4 Gelatinization characteristics of flours and starches.

Sample	Temperature (°C)[1] T_o	T_p	T_c	Heat of transition (J/g starch) ΔHg
Five Roses flour	55.5±0.7	64.1±0.3	83.0±1.5	11.6±0.4
Five Roses starch	53.2±0.3	60.2±0.3	79.2±1.4	12.8±0.9
Primrose flour	55.3±0.4	62.6±0.5	81.6±0.2	13.1±0.6
Primrose starch	53.8±0.3	59.9±0.3	78.0±1.8	12.7±0.8

[1] Mean ± S.D.

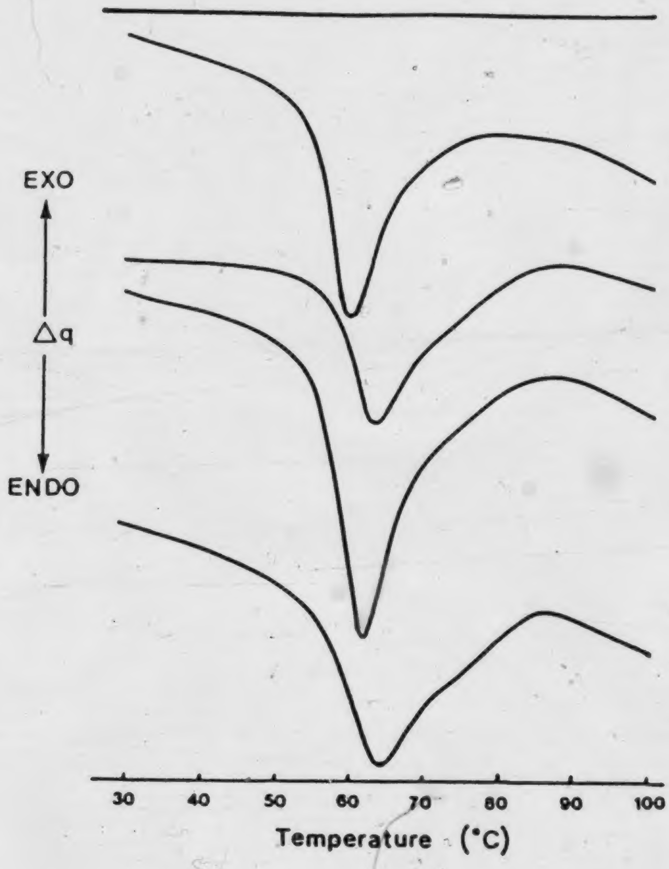


Figure 4.6 DSC thermograms of flours and starches. a. Primrose starch; b. Primrose flour; c. Five Roses starch; d. Five Roses flour.

Primrose flour cannot be considered different from ΔHg for Primrose starch, whereas Five Roses flour exhibited a slightly reduced ΔHg when compared to its isolated starch.

Stevens and Elton (1971) considered the possibility that in the application of DSC analysis to wheat flour an endotherm due to protein denaturation may be superimposed on the gelatinization peak. They carried out experiments using aqueous solutions of proteins and obtained immeasurably small peaks. Thus, they concluded that the main effect of the presence of protein on the starch gelatinization endotherm may be expected to be merely that of a diluent. However, Berry and White (1966) have shown that the gluten of wheat flour increases the gelatinization temperature of wheat starch. Eliasson (1983a) found that both T_p and ΔHg are dependent on the ratio of gluten to starch. She found that gelatinization temperature increased as the ratio of gluten to starch increased, while ΔHg (in J/g of starch) decreased. Since these effects of gluten on starch gelatinization are similar to those caused by reducing the water content, Eliasson (1983a) interpreted the results on the basis of competition for available water by gluten.

Stevens and Elton (1971) found that the presence of damaged granules in a starch sample resulted in a reduction of ΔHg, such that a completely damaged sample gave an immeasurably small endotherm. ΔHg for Five Roses flour was slightly less than ΔHg for Five Roses starch, which may be related to damage levels as well as the gluten effect. ΔHg for Primrose starch and flour was approximately equal, which may be expected since starch damage levels were relatively low and protein content was less than that of Five Roses flour.

The range of values found for ΔHg in the present study (Table 4.4) compares well with the 10.1-12.2 J/g starch reported by Stevens and Elton (1971), 12.6 J/g starch by Gough and Pybus (1971) and 9.2-10.1 J/g starch by Lelièvre (1975). Wootton and Bamunuarachchi (1979) published a value of 19.7 J/g starch for a slurry of wheat starch at a ratio of water to dry starch of 2:1 which is considerably higher than other reported values.

4.2 Emulsifier Phase Transitions

Thermally-induced transitions were studied using DSC analysis. The results are shown in Table 4.5 and Figures 4.7 and 4.8. For convenience the DSC thermograms are shown with temperature on the x-axis. T_p of the MG endotherm is in agreement with specifications given by the manufacturer detailing a melting point of 67°C. The MG phase transition occurred over a range of about 10 C°. Since the MG used in the present study was actually a mixture of monoglycerides with different fatty acid moieties, data obtained cannot be compared exactly with literature values for pure compounds. The heat of melting obtained for MG of ca. 150 J/g is similar to the 142 J/g given by Lutton (1971) for melting of the α-form of 1-monostearin and the 160 J/g found by Cloke

Table 4.5 Characteristics of thermal transitions of MG and SSL.

	Temperature (°C)[1]			
Sample	T_o	T_p	T_c	Heat of transition (J/g emulsifier)
MG	60.5±0.9	68.2±0.9	70.9±0.3	151.7±4.7
MG'[2]	15.4±0.1	20.7±0.9	24.6±0.9	13.1±0.9
	47.6±1.0	58.9±0.3	61.9±0.4	66.1±1.5
SSL[3]	43.5±0.3	48.6±0.6	52.1±0.3	91.0±4.3
SSL'	42.5±0.6	47.2±0.3	51.2±0.6	92.4±3.7
MGaqu[4]	46.2±0.6	52.9±0.3	55.9±1.0	119.7±6.2
MGaqu'	36.4±3.1	47.5±0.0	52.5±0.5	45.5±1.3
SSLaqu[4]	48.4±0.2	50.7±0.0	54.1±0.5	57.9±2.4
SSLaqu'	47.3±0.4	49.8±0.4	53.0±0.0	54.0±1.1

[1] Mean ± S.D.

[2] MG' signifies MG reheated.

[3] SSL scans also showed immeasurably small peaks at 69°C.

[4] aqu signifies emulsifier with water either as dispersion form or added in the sample pan.

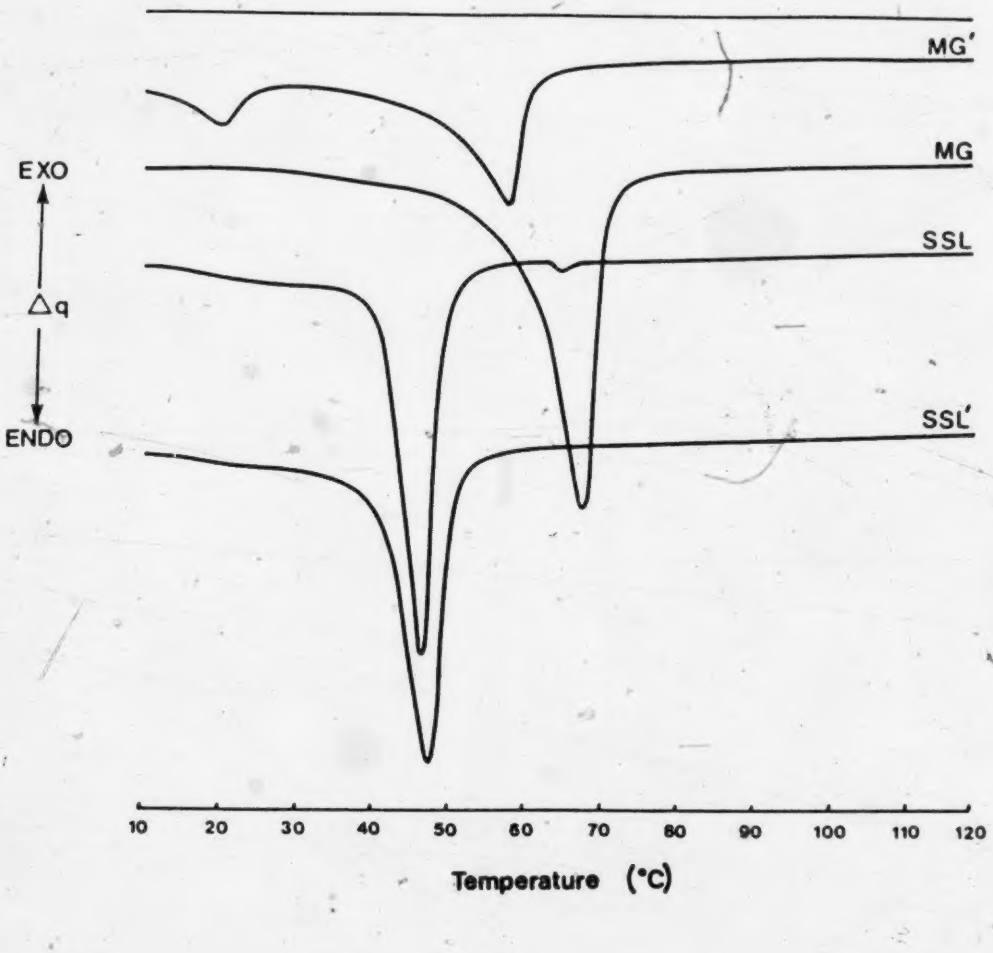


Figure 4.7 DSC thermograms of emulsifiers. MG; MG' (MG reheated); SSL; SSL' (SSL reheated).

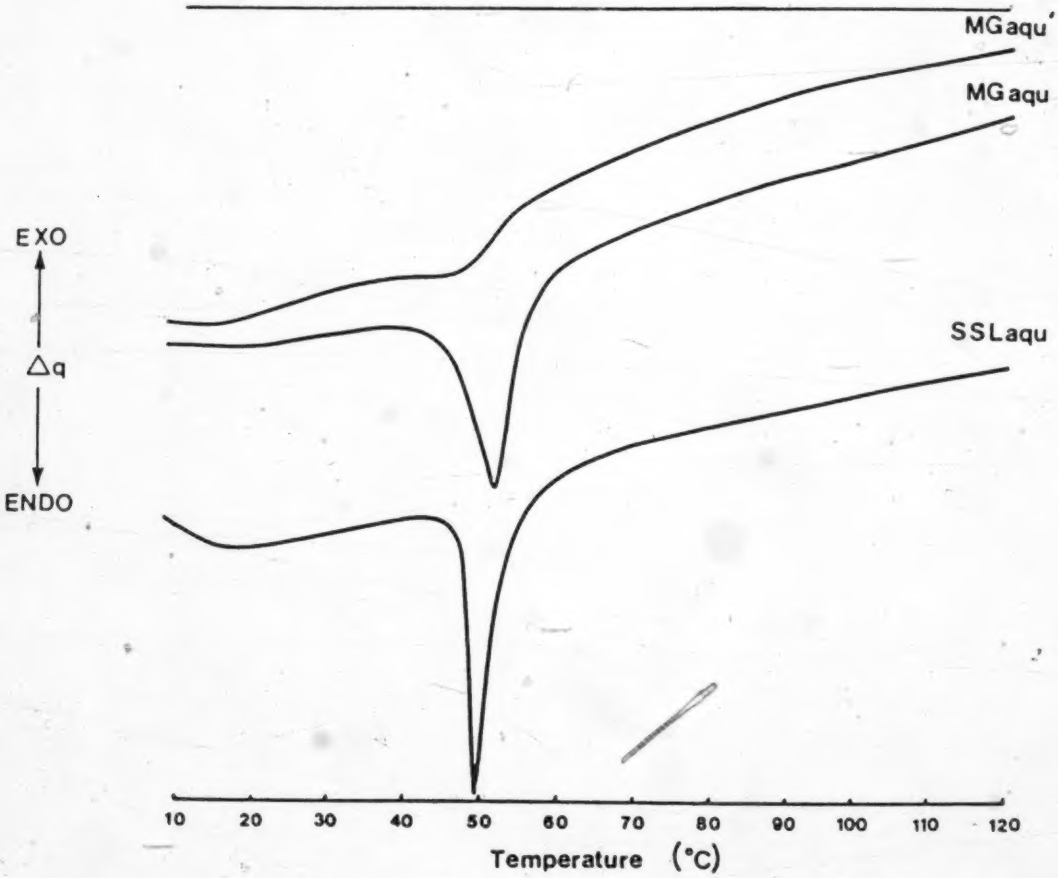


Figure 4.8 DSC thermograms of emulsifiers in presence of water. MGaqu; MGaqu' (MGaqu reheated); SSLaqu (SSLaqu reheated was unchanged).

et al. (1983) for a commercial emulsifier containing 85-90% monostearin. On reheating, MG exhibited two thermal transitions at different temperatures from the original and with different enthalpies. The mean heat of transition for the low temperature endotherm was 13.1 J/g, which is similar to values reported by Lutton (1971) for the sub α_2 to sub α_1 transformation of $C_{18:0}$ to $C_{22:0}$ 1-monoglycerides. However, the T_p of 20.7°C is much lower than those given by Lutton (1971) of between 40 and 50°C. Transition points of 1-monoolein were given by Bailey (1950) as 35.0, 32.0, 25.0 and 12.5°C depending on the crystal form. MG in the present study contained 42% monoolein, 44% monostearin and minor amounts of other MGs. Thus it is likely that the transition characteristics of the MG of the present study are mainly due to the influences of monostearin and monoolein. Therefore, the low temperature transition obtained on reheating may be due to the presence in the mixture of monoolein. The second transition during the reheating run had an enthalpy similar to those reported for the sub α_1 to α transformation of various monoglycerides (Lutton, 1971); T_p was also within the reported range of 46 to 64°C. It would appear then that the MGs comprising the commercial MG used in the present study are predominantly in the α-form.

SSL showed a thermally-induced phase transition occurring over a range of about 8 C° between 43.5 and 52.1°C with mean T_p equal to 48.6°C, which agrees with the manufacturer's specifications of a melting point between 47 and

52°C. On reheating, characteristic temperatures were reduced slightly while the enthalpy of transition was unchanged. This indicates that SSL does not exhibit polymorphism to the extent of that shown by MG. Initial heating of SSL also gave a small endothermic peak at 69°C which did not appear on reheating. The melting point of stearic acid is 69°C (Bailey, 1950), so this transition was attributed to the presence of a small amount of stearic acid in excess of requirements for the reaction between stearic and lactic acids used to produce SSL.

The thermal characteristics of the emulsifiers were changed when heated in the presence of water (MGaqu and SSLaqu in Table 4.5). However, the method of addition of the water did not affect the results. The MGaqu transition on initial heating occurred at a lower temperature and with less energy involvement than that for MG. On reheating, the transition temperature was even lower and the enthalpy was about half of that for the initial transition. Water dissolves only a fraction of a percent of MG but does incorporate it and lowers its melting point (Lutton, 1965).

SSLaqu exhibited an endotherm occurring over a range at higher temperatures and with smaller enthalpy than for SSL melting. However, the temperature shift was not as great as in the MG case. There were no significant differences between the thermal characteristics of the initial SSLaqu and the second SSLaqu (SSLaqu') scans. Krog (1981) has described the formation of lyotropic, mesomorphic phases when surfactant crystals are mixed with water and this phenomenon may result in the different thermal characteristics observed after the addition of water.

4.3 Batter Phase Transitions

The heating program for the "cooking" scans was designed to imitate the temperature regime experienced by the mid-portion of the crumpet during cooking. The actual temperature profile of a crumpet during cooking from the underside is shown in Figure 4.9.

Results of the DSC studies of crumpet batter Trials I to III are given in Figures 4.10 and 4.11 and Tables 4.6, 4.7 and 4.8. Table 4.6 shows the starch gelatinization temperatures T_o and T_p obtained during the DSC "cooking" scans. T_c is not given since it was very close to the upper limit of the scan and could not be accurately determined. Heats of transition are given in J/g of carbohydrate (J/g CHO) which, since starch comprises the major part of the carbohydrate content, can be considered approximately equivalent to J/g starch. Gelatinization endotherms were essentially unchanged whether the emulsifiers were added in powder or dispersion form. Figure 4.10 shows typical endotherms obtained during the DSC "cooking" scans. On several of the DSC thermograms of batter containing an emulsifier an immeasurably small endotherm was observed due to the melting of the SSL or MG. Figure 4.11 shows the representative DSC thermograms of the batters when heated

Table 4.6 Characteristics of thermal transitions of batters during a model cooking process.

Sample	Temperature (°C)[1] T_o	T_p	Heat of transition (J/g CHO)[2]
Control	64.0±0.7	72.1±0.4	10.7±0.7
SSL	65.1±0.8	73.1±0.8	9.2±0.7
MG	63.7±0.3	72.0±0.3	10.4±0.4

[1] Mean±S.D.
[2] Heat of transition units: J/g carbohydrate.

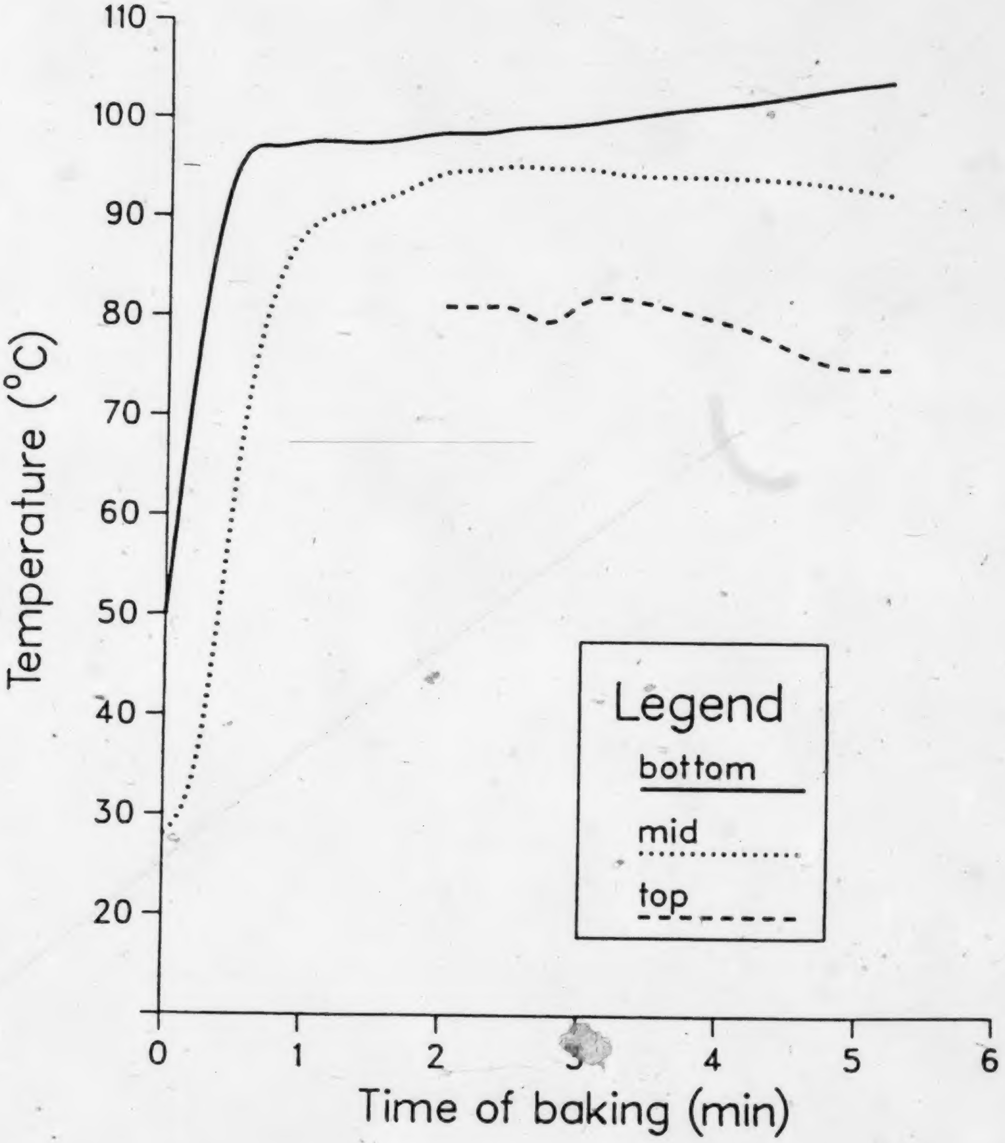


Figure 4.9 Temperature profile of a crumpet during cooking.

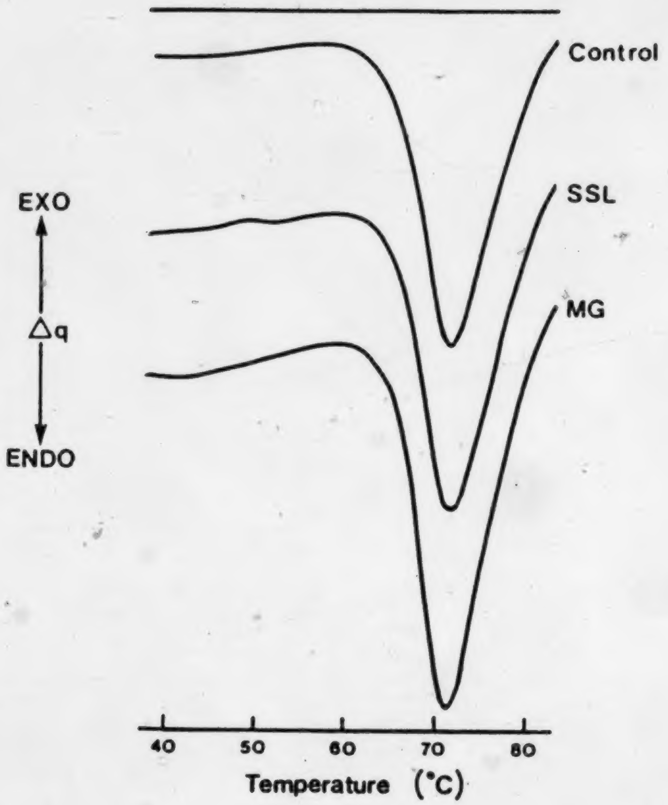


Figure 4.10 DSC thermograms of batter during "cooking" regime (heating rate 20 C°/min).

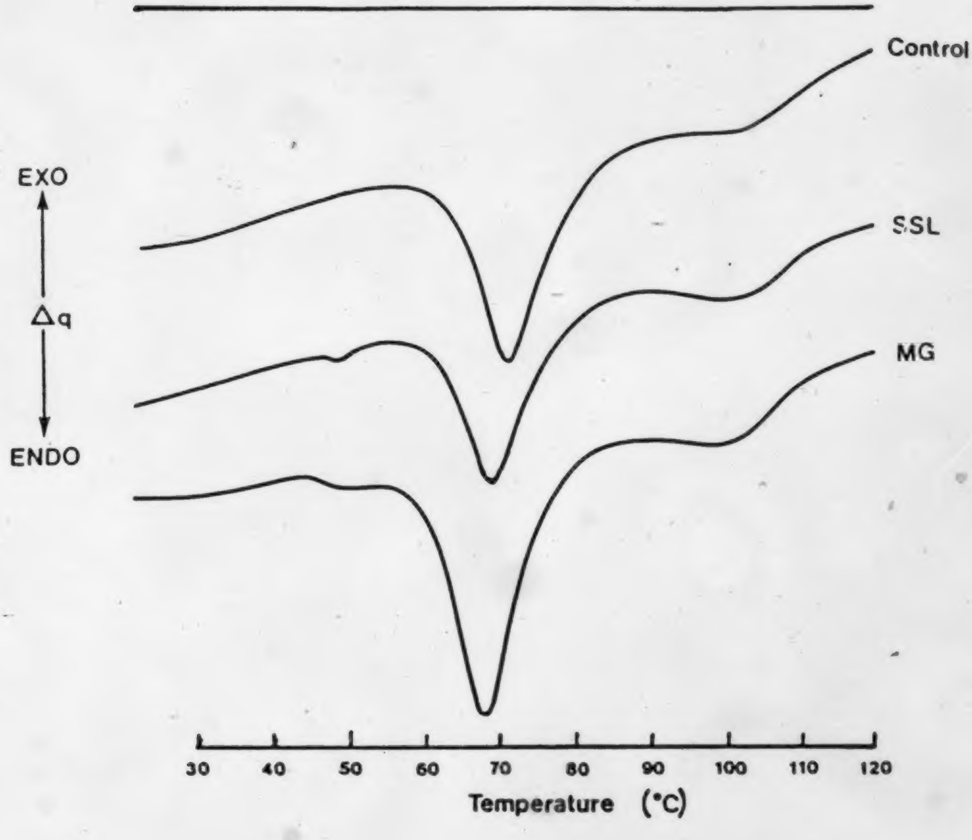


Figure 4.11 DSC thermograms of batters (heating rate 10 C°/min).

between 7 and 125°C at 10 C°/min. Characteristics of the endotherms are detailed in Table 4.7.

Trial III involved addition of the emulsifiers at a higher level than in the previous two trials. The two emulsifiers were added at a level of 0.5% of flour weight both in powder and dispersion form. Table 4.8 shows the results of DSC analysis. The batters containing emulsifiers all yielded very small endotherms due to emulsifier transitions. The characteristic temperatures of these endotherms are given in Table 4.8 but the heats of transition were not determined.

It can be seen from the characteristics of the gelatinization endotherms obtained using a heating rate of 10 C°/min, shown in Tables 4.7 and 4.8, that the presence of the non-flour batter ingredients had the effect of moving the transition to a higher temperature than that for the flour-water slurry (Table 4.4). There is also some evidence of a narrowing of the temperature range over which the transition occurred. Wootton and Bamunuarachchi (1980) found that addition of sucrose to a starch-water slurry increased T_p while T_o and T_c were unaffected. In the same study, it was noted that addition of salt up to a 9% level in the aqueous phase increased T_o, T_p and T_c. With both solutes gelatinization energy was observed to decrease.

Temperatures at which starch gelatinization occurred were observed to be dependent on heating rate. T_o and T_p determined using a scan with a heating rate of 20 C°/min

Table 4.7 Characteristics of thermal transitions of batters in Trial II.

Sample	Temperature (°C)[1] T_o	 T_p	 T_c	Heat of transition (J/g CHO)
Gelatinization				
Control	61.7±0.1	69.7±0.6	78.3±1.0	10.2±0.6
SSL	60.7±0.4	68.7±0.4	78.0±1.2	10.9±1.2
MG	61.4±0.4	69.1±0.6	78.1±0.9	10.7±0.7
Amylose-lipid complex				
Control	90.2±1.0	102.8±1.1	111.6±1.2	1.7±0.1
SSL	89.4±0.2	103.9±0.4	112.3±0.4	2.8±0.6
MG	94.1±0.9	105.4±1.0	112.6±0.7	2.4±0.1

[1] Mean±S.D.

Table 4.8 Characteristics of thermal transitions of batters in Trial III.

Sample	Temperature (°C)[1]			Heat of transition (J/g CHO)
	T_o	T_p	T_c	
Emulsifier melting				
Control	---[2]	---[2]	---[2]	---[2]
SSL(P)[4]	46.5±0.4	48.6±0.4	51.3±0.5	---[3]
SSL(D)[5]	45.1±0.9	47.2±0.9	49.8±0.5	---[3]
MG(P)	48.1±1.1	51.7±0.6	54.3±0.9	---[3]
MG(D)	48.6±0.6	52.4±0.5	55.7±0.7	---[3]
Gelatinization				
Control	61.2±0.5	69.0±0.0	77.2±0.0	11.2±0.7
SSL(P)	61.5±0.0	69.2±0.3	77.3±0.5	10.5±0.4
SSL(D)	61.3±0.3	69.1±0.2	76.9±0.4	10.3±0.4
MG(P)	61.0±1.0	69.0±0.0	76.8±0.7	10.2±0.3
MG(D)	61.0±0.6	68.8±0.4	77.4±0.3	12.0±2.5
Amylose-lipid complex				
Control	89.1±0.9	102.6±0.3	110.4±0.5	*1.5±0.2[6]
SSL(P)	89.9±1.3	103.0±0.5	110.5±0.0	*1.7±0.2
SSL(D)	90.3±0.9	102.8±0.7	109.6±0.8	**2.7±0.4
MG(P)	90.3±1.5	102.3±0.4	109.9±0.8	*1.6±0.4
MG(D)	90.8±0.5	103.4±0.5	110.9±1.0	**2.3±0.2

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[1] Mean±S.D.

[2] Not applicable.

[3] Not determined.

[4] (P) indicates emulsifier added in powder form.

[5] (D) indicates emulsifier was added in dispersion form.

[6] Means bearing one asterisk are significantly different from means bearing two; Student-Newman-Keuls' test, $\alpha=0.01$.

were higher than the T_o and T_p of a scan with heating rate of 10 C°/min. Wootton and Bamunuarachchi (1979) used DSC to study the effect of heating rate on starch gelatinization. They found that increasing the heating rate from 8 to 32 C°/min caused T_o to decrease from 52 to 46°C, T_p to change from 67 to 65°C and T_c to increase from 78 to 85°C. The heat of gelatinization decreased with increasing heating rate. Therefore, these workers concluded that the general effect of increasing heating rate on gelatinization was a broadening of the endothermic range. Researchers using DSC to study thermal denaturation of proteins have found that T_p of the denaturation endotherm increased as the heating rate increased while T_o was fairly insensitive (Donovan and Ross, 1973; de Wit and Swinkels, 1980). This was attributed to the fact that the rate of protein denaturation was comparable to the programming rate. Clearly, this reasoning cannot be directly applied to the present study since both T_o and T_p were increased (T_c at 20 C°/min was not determined). This phenomenon emphasizes the necessity to compare only endotherm characteristics obtained using identical instrumental conditions.

It is apparent from Tables 4.6, 4.7 and 4.8 that addition of SSL and MG at any of the levels used, in either powder or dispersion form, had no effect on the gelatinization of starch in the model batters as determined using DSC. Eliasson (1983b) studied gelatinization of a gluten-starch-water mixture at a ratio of 0.2:1:0.9. SSL was added in dispersion form at two levels: 0.006 g SSL/g starch and 0.023 g SSL/g starch. She found that the gelatinization endotherm, under these conditions where water was limited, was delayed by SSL. The upward temperature shift of gelatinization increased with increasing amount of SSL.

In the DSC scans which involved heating above 100°C, an endotherm due to breakdown of the amylose-lipid complex was observed. Bulpin *et al.* (1982) reported an enthalpy change of 1.7 J/g maize starch for this endotherm; Kugimiya *et al.* (1980) determined a value of 2.1 J/g wheat starch. Both of these results were determined by DSC using a heating rate of 10 C°/min using dilute samples (ca. 25% and 16% w/w starch, respectively). Eliasson (1983b) detailed a value of 2.4 J/g starch for this transition in a gluten-wheat starch-water mixture. Values obtained in the present study and shown in Tables 4.7 and 4.8 for the control batters are in agreement with these published data. It was found (Eliasson, 1983b) that addition of SSL at the high level of 2.3% of starch weight caused the enthalpy change involved with the melting of the amylose-lipid complex to increase to 4.6 J/g starch. Russell (1983b) found that addition of monostearin to bread dough caused an increase in the heat of melting of the complex, indicating incorporation of the monoglyceride in the amylose. Results in Tables 4.7 and 4.8 indicate that addition of emulsifiers had no effect on the temperature at which the upper endotherm occurred. Trial II data suggest that addition of MG and SSL at 0.14% and 0.3% of flour weight respectively in dispersion form did have an effect on the size of the heat of transition involved with this endotherm. Trial III was designed to show the influence of form of emulsifier added to the batter on its ability to complex with amylose. The mean values of heat of melting of the amylose-lipid complex were compared using a Student-Newman-Keuls' test. It is apparent that addition of MG and SSL in powder form had no effect on the size of the endotherm over that of the control batter. However, values for both MG and SSL added in dispersion form indicate a significant effect on the size of the endotherm. Thus, these data suggest that addition of these two emulsifiers in dispersion form facilitates complexation with the amylose, while their addition in powdered form will not.

Kugimiya *et al.* (1980) conducted experiments with potato starch and lysolecithin and deduced that a complex was formed exothermically simultaneously with gelatinization since that endotherm was decreased in size by an amount equivalent to the complex-melting endotherm. In the same study, a similar experiment with palmitic acid yielded no complex formation, which was attributed to the fact that, unlike lysolecithin, palmitic acid is not water soluble. Results obtained by Kugimiya and Donovan (1981) from studies of interaction of lysolecithin with several different starches agreed with this hypothesis of an exothermic formation of the amylose-lipid complex at gelatinization temperatures.

Cloke *et al.* (1983) studied thermal transitions in model cake systems containing both saturated and unsaturated MGs. They did not detect measurable complexing of emulsifier with starch in the temperature range that Kugimiya and Donovan (1981) reported for complex formation and melt. The systems used in the studies of Cloke *et al.* (1983) contained high proportions of sucrose which is known to have an effect on the gelatinization of starch (Spies and Hoseney, 1982; Wootton and Bamunuarachchi, 1980). Cloke *et al.* (1983) concluded that the starch:sucrose:water ratios of the cake batters may have caused a shift of the amylose-lipid endotherm to temperatures higher than those reached during the DSC scans.

In the present study, there is no evidence to suggest that the starch gelatinization endotherm was reduced in size by addition of MG or SSL in either form, even when the size of the melting endotherm indicated complex formation. It is possible that there was an exothermic formation of the complex during gelatinization which did not register on the DSC thermograms under the experimental conditions used. A major complicating feature may be the presence of other components in the samples, whereas Kugimiya *et al.* (1980) were working with the more simple starch-water-lipid system.

4.4 Physicochemical Characteristics of Crumpets and Batters

4.4.1 Moisture contents and pH of crumpets and batters

The moisture contents and pH values of the crumpets and batters are shown in Table 4.9. It can be seen that the moisture content of the final product is somewhat greater than that of bread (≃36%, wet basis (Ponte, 1971)). The pH is low ~~in~~ order to assist in the extension of microbiologically safe shelf-life of the product; however, this pH value is in the range found by Kalb and Sterling (1962) to promote maximum retrogradation of corn starch.

4.4.2 SEM of crumpets and batters

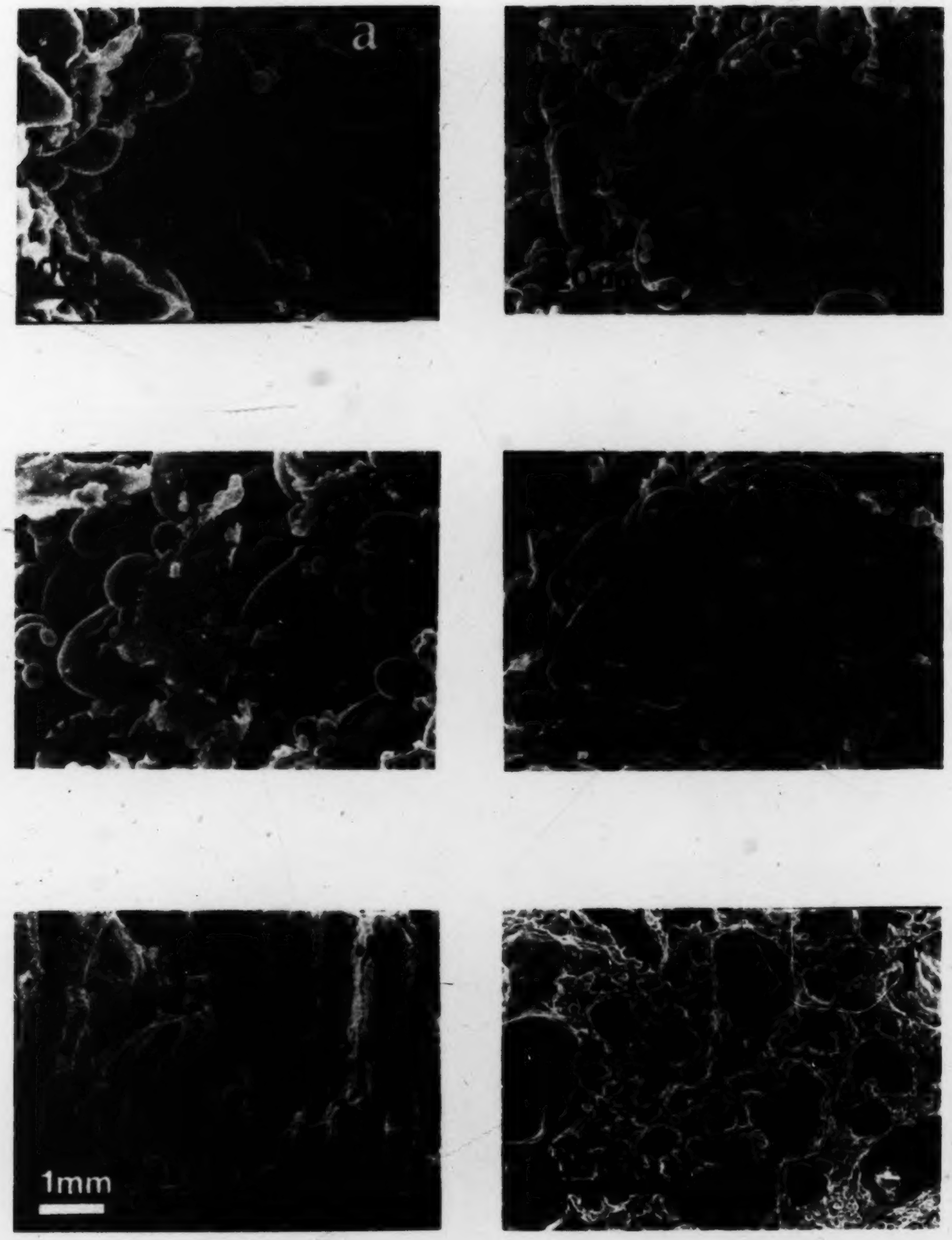
Scanning electron micrographs of crumpet batter are presented in Plate 4.2 (a,b,c,d). Micrographs (a) and (b) are of control batter; the sample shown in (a) was prepared by vacuum desiccation while that shown in (b) was freeze dried prior to microscopy. It appears that both methods gave similar results, thus the opinion of Varriano-Marston (1977) that these two methods are excellent SEM preparation procedures for bread dough can be extended to low-fat batters. Plate 4.2 (c) and (d) show SSL and MG batters, respectively, prepared by freeze drying. It was not possible to differentiate between control, SSL and MG batters from their microstructure as revealed by SEM. Most of the starch granules are free of any restrictive matrix, although protein is still evident in a similar form as seen in the

Table 4.9 Moisture contents and pH of crumpets and batters.

	Moisture content' (% wb)	pH'
Crumpet	53.84±0.65	5.46±0.03
Batter	60.88±0.08	5.58±0.02

' Mean of triplicates ± S.D.

Plate 4.2. SE-Micrographs of batters and crumpets. (a) control batter, vacuum desiccated; (b) control batter, freeze dried; (c) SSL batter, freeze dried; (d) MG batter, freeze dried; (e) crumpet, longitudinal fracture; (f) crumpet, lower surface.

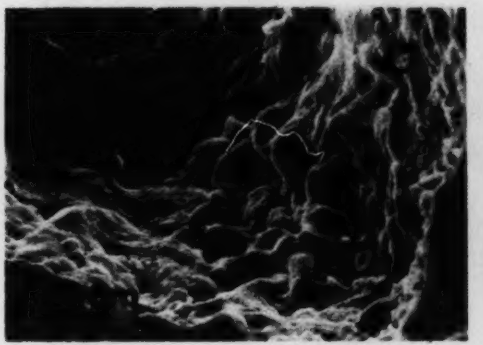

a
1mm

flour particle. The batter structure contrasts markedly with that of a bread dough where the gluten has been developed and holds the starch granules together with its sheets, strands and filaments (Angold, 1979).

For crumpet manufacture, the batter is poured onto a hot plate, with heat being applied only from the underside. Steam and carbon dioxide escape from the baking product and form the characteristic crumpet structure. Plate 4.2 (e) shows a crumpet that has been fractured longitudinally to reveal the channels produced during baking, while 4.2 (f) is a transverse view of the lower surface of the crumpet. Some of the channels run from the lower surface to the top while others are blocked off.

Plate 4.3 shows SE micrographs of control crumpets. (a) and (b) were prepared by vacuum desiccation while (c) and (d) were freeze dried. The surface of the wall ('w') of one of the longitudinal channels can be seen in (a). There appears to be either a protein coat through which the shape of starch granules can be seen or starch granules and protein fused together at the surface. The sample was fractured to reveal the interior where the starch granules are twisted and deformed. Micrograph (b) again shows the interior of the crumpet. In the centre there can be seen a lenticular granule in a saddle shape which has been shown by Bowler *et al.* (1980) to occur when starch is heated in water to 70°C. Micrographs (c) and (d) are of freeze dried samples of control crumpet. Both show the surface of a channel; (c)

Plate 4.3. SE-Micrographs of control crumpets. (a),(b) vacuum desiccated, 'w' indicates surface of wall; (c),(d) freeze dried.

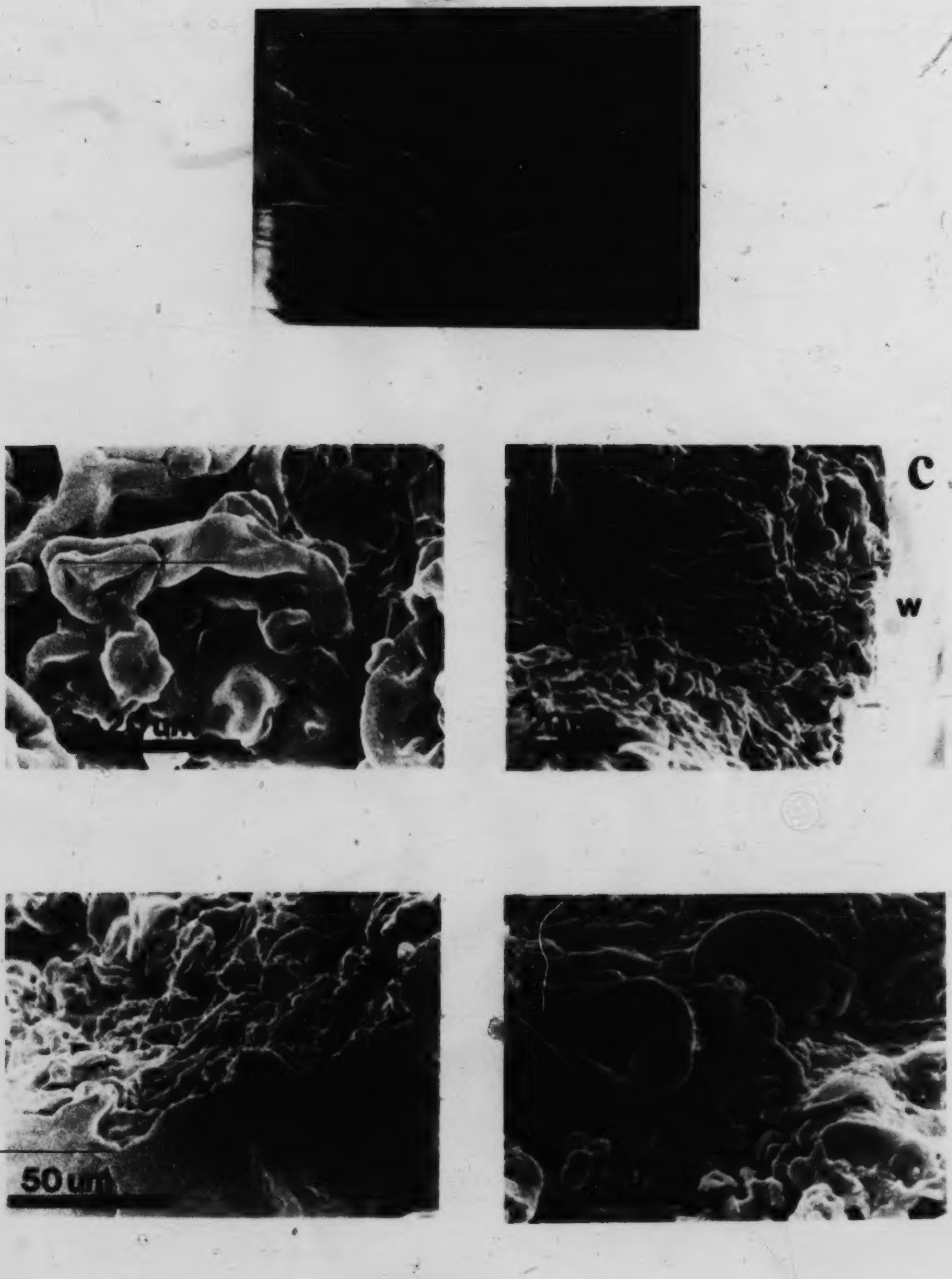


is located close to the lower surface of the crumpet while (d) is in the central region. It can be seen that while a thorough coating on starch granules can be achieved, as in (d) and parts of (c), there are also regions where starch granules are clearly discernible and appear essentially free. Micrograph (c) is somewhat similar to one published by Angold (1979) of wheat flour bread baked from grossly overmixed dough in which many of the starch granules were not enmeshed in the gluten film.

Plate 4.4 shows micrographs of crumpets containing SSL and MG. (a), (b) and (c) are of SSL-containing crumpet; (a) was prepared by freeze drying, (b) and (c) by vacuum desiccation. Micrograph (a) shows the surface of an air hole in the interior of the product. The starch granules appear somewhat fused together and coated with protein. (b) and (c) show the interior of the crumpet. The surface of a channel ('w') can be seen in (c) with very tightly packed starch granules comprising the interior. Plate 4.4 (d) and (e) are micrographs of MG-containing crumpets prepared for SEM by vacuum desiccation; (d) is similar to micrograph (c). The coating ('w') shown in (d) is rather thick and it is difficult to make out the shape of the starch granules beneath. Plate 4.4 (e) shows an air-hole surface; some starch granules appear firmly held while others are relatively free.

It was very difficult to observe any differences that may have been made to the crumpet structure by addition of

Plate 4.4. SE-Micrographs of SSL- and MG-crumpets. (a) SSL, freeze dried; (b),(c) SSL, vacuum desiccated; (d),(e) MG, vacuum desiccated. 'w' indicates surface of wall.


20 um
C
w
20
50 um

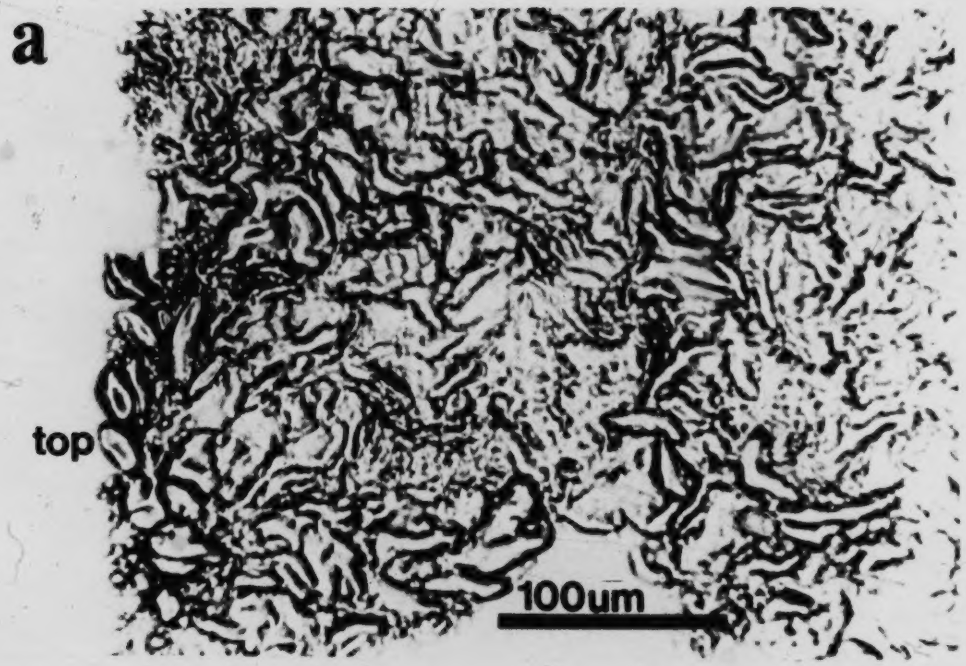
emulsifiers.

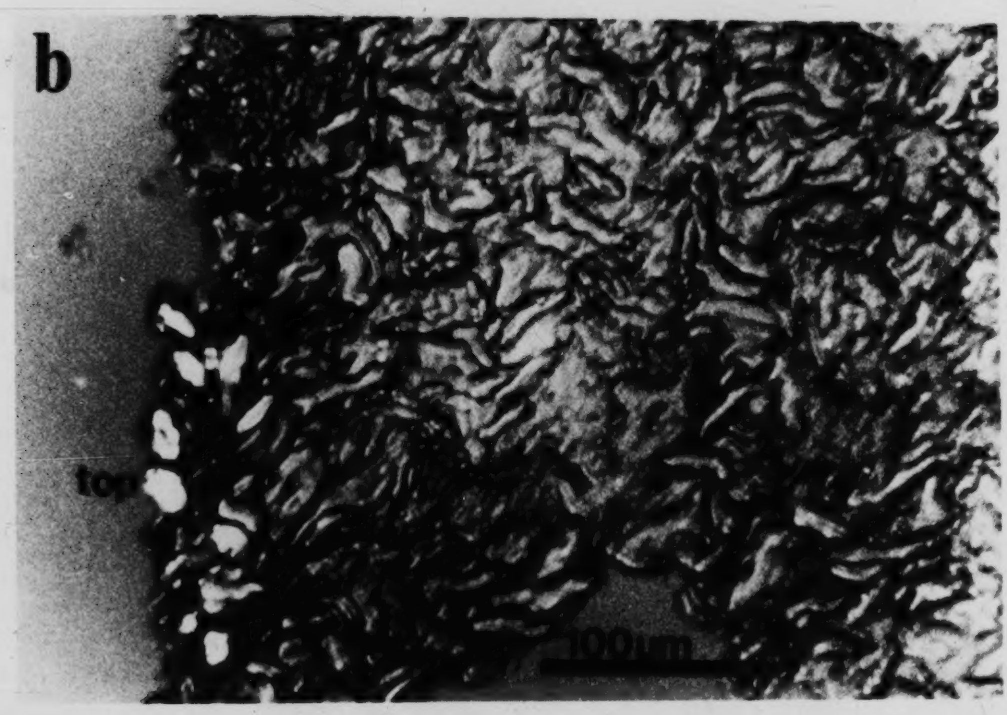
In an SEM study on the structure of bread dough by Evans *et al*. (1977) it was found that addition of SSL improved the sheeting action of gluten during dough formation. However, that research was not extended to the effects of SSL on bread structure. Khoo *et al*. (1975) carried out SEM on bread and were able to identify stretched protein but it was not stranded as clearly as in the dough. They noted that the protein veil, which was sheeted in the dough, formed a cohesive mass with the underlying starch. This is similar to the appearance of the channel wall surface in the present study. However, it has been shown that the crumpet interior is made up of deformed starch granules packed tightly together with no evidence of an interlinking protein network.

4.4.3 Light microscopy of crumpets

Plate 4.5 shows an unstained crumpet section taken from the top part of the crumpet. In micrograph (a) the outline of densely packed starch granules can be seen. When the same field was viewed through polarized light, micrograph (b) was the result. The majority of the starch granules at the upper surface of the crumpet exhibited birefringence while those in the interior did not. It was apparent that the birefringent granules had retained their characteristic shape while the others, although still discernible, were stretched and deformed. The starch granules at the upper

Plate 4.5. L-Micrographs of crumpet section. (a) unstained section including top surface; (b) same field using polarized light.


a
top
100um


b
top
100um

surface were not gelatinized during baking due to low temperatures and limiting water content in this region.

Plates 4.6, 4.7 and 4.8 show crumpet sections having been stained using the periodic acid Schiff (PAS)/Fast Green procedure. Plate 4.6 shows a transverse section through a control crumpet, 4.7 shows a transverse section through a crumpet containing SSL and 4.8 shows a longitudinal section through an MG-containing crumpet. It was apparent that addition of emulsifier had no effect on the microstructure as seen using this staining procedure. It can be seen that the protein (stained green) does not form a continuous network throughout the crumpet, rather, it is present as masses entrapping gelatinized starch granules. The main body of the product appears to be made up of tightly packed starch granules. Plate 4.8 shows the surface wall ('w') of one of the vertical channels as seen in the SE-micrograph (Plate 4.2e). It can be seen that the starch granules are looser in that area. These granules were probably freed during the slide preparation procedure since they were not held in place by protein.

Plates 4.9, 4.10 and 4.11 show crumpet sections stained only with Fast Green to better show the location of protein. Plate 4.9 shows a longitudinal section of a control crumpet. The upper surface wall of a longitudinal channel is marked ('w') and the top surface of the crumpet can be seen ('t'). Both the lenticular A-granules and the smaller B-granules are discernible at the upper surface of the crumpet; both

Plate 4.6 Control crumpet, transverse section, **PAS/Fast** Green stain (bar indicates 100 μm).

Plate 4.7 Crumpet containing SSL, transverse section, PAS/Fast Green stain (bar indicates 100 µm).

Plate 4.8 Crumpet containing MG, longitudinal section, PAS/Fast Green stain (bar indicates 100 μm).

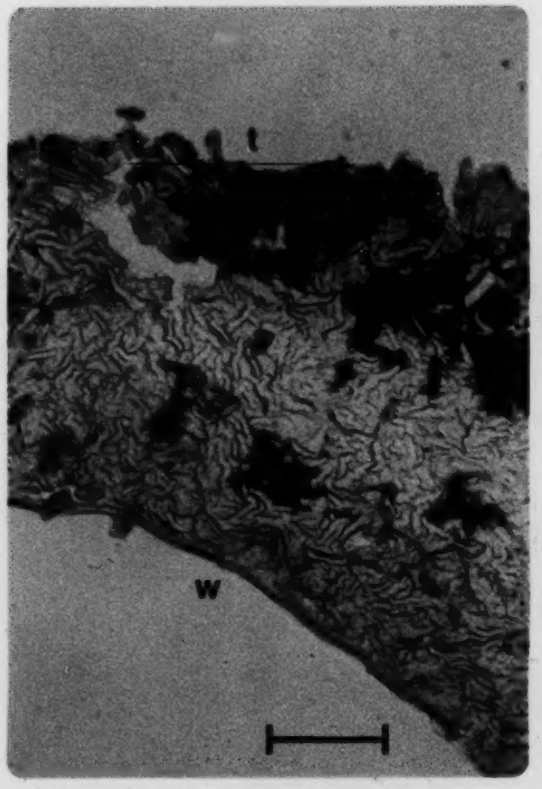


Plate 4.9 Control crumpet, longitudinal section, Fast Green stain (bar indicates 100 µm).

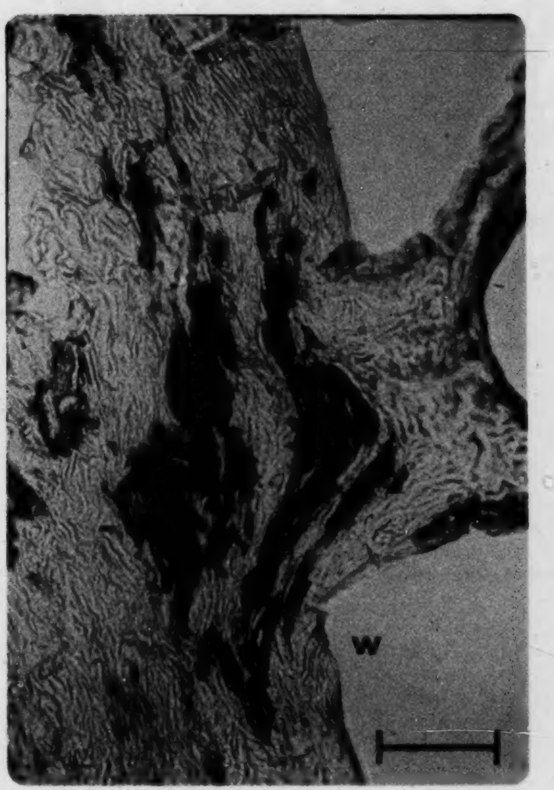


Plate 4.10 Control crumpet, longitudinal section, Fast Green stain (bar indicates 100 μm).

Plate 4.11 Crumpet containing SSL, transverse section, Fast Green stain (bar indicates 100 μm).

types appear unswollen. These are the granules which were seen to exhibit birefringence as shown in Plate 4.5b. In the interior of the crumpet it was impossible to differentiate the two types of starch granules, they appeared as an intertwined stretched and deformed mass. Again, the protein appeared irregularly dispersed as masses.

Plate 4.10 shows a longitudinal section through a control crumpet. It can be seen that the protein mass has been stretched out and is aligned parallel to the surface wall of the longitudinal channel ('w'). The starch granules have also been stretched and pulled in line with the forces exerted during the raising action on baking.

Plate 4.11 shows a transverse section through a crumpet containing SSL. A protein mass ('m') can be seen next to a much more diffuse protein arrangement ('d'). This diffuse area has the appearance of a form of protein network containing gelatinized starch granules.

Plate 4.12 shows a transverse section through a crumpet containing SSL stained using Gram's Iodine. Sections through control and MG-containing crumpets and iodine-stained appeared similar. Small protein masses can be seen stained yellow as described by Flint and Johnson (1979) while starch granules are stained blue-purple.

It was apparent from light microscopy that crumpets do not contain a protein network of the kind reported in doughs (Moss, 1972; Angold, 1979). Moss (1972) studied the microstructure of doughs and found that dough mixing destroyed

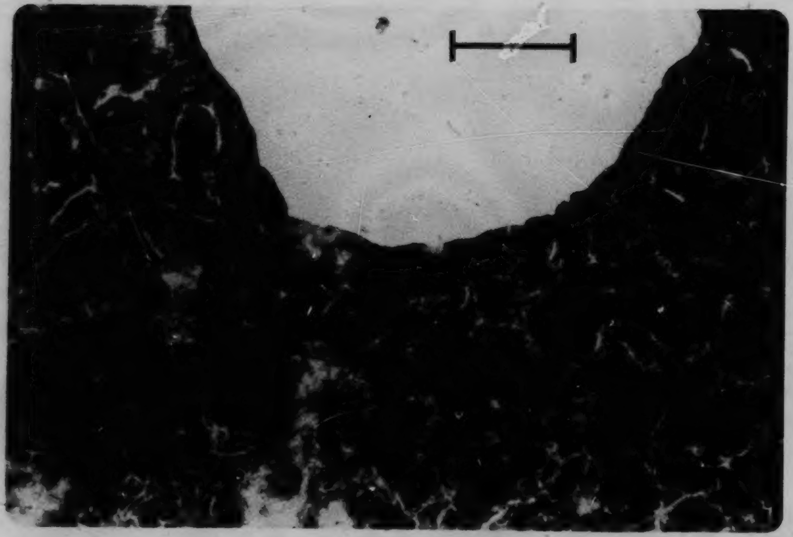


Plate 4.12 Crumpet containing SSL, transverse section, iodine stain (bar indicates 100 µm).

the protein matrices present in the flour particle and developed the gluten into large masses. On further mixing, the protein masses were stretched out to form a continuous network which then formed a mantle surrounding all the starch granules. In the present study it was observed that most of the protein was present as large masses with swollen and deformed starch granules tightly packed throughout the crumpet. Protein masses and starch granules were seen to be stretched and aligned near channel surfaces due to forces occurring during baking. Such stretching and alignment of starch granules has been noted previously by Dennett and Sterling (1979). It appeared from the PAS/Fast Green stained sections that the walls of the channels are not coated with protein as may have been concluded from SE-micrographs; selective staining points to the surface being made up of starch.

4.5 Storage Trials

4.5.1 Short-term storage

Changes in the starch fraction of the cooked model batters during storage in the DSC pan for up to 6 days at 5 and 25°C were studied by using DSC to follow development of the staling endotherm. Figure 4.12 shows the typical development of the staling endotherm during storage. The endotherm present in scan 'b' at the lower temperature is the staling endotherm; that at the higher temperature and

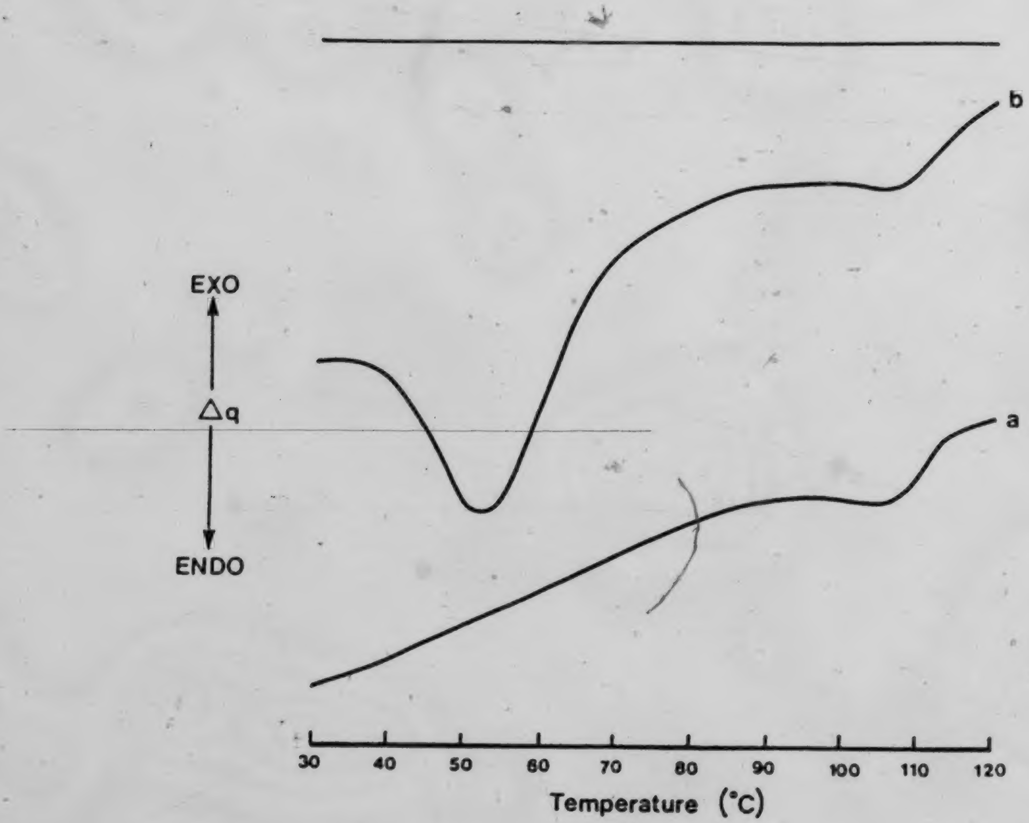


Figure 4.12 Development of staling endotherm on storage. a. fresh product; b. product after storage.

present in both 'a' and 'b' scans is the amylose-lipid endotherm. Scan 'a' represents the thermogram obtained from a freshly prepared baked product; scan 'b' represents that obtained from a sample of the same product after storage. The staling endotherm was found to increase in size during storage while, in accordance with previous reports (Russell, 1983b,c), length and temperature of storage had no effect on the size or characteristic temperatures of the amylose-lipid endotherm. Table 4.10 shows the data obtained on the latter endotherm during storage trials I and II. Means and S.D.'s were calculated using all data, regardless of temperature of storage or number of days stored. The characteristic temperatures of the amylose-lipid endotherm determined during storage trials I and II were the same, i.e. $T_0 \simeq 88°C$; $T_p \simeq 103°C$; $T_c \simeq 111°C$. However, when these are compared to the temperatures of the amylose-lipid melting endotherm determined from the gelatinization scans of the batters (Tables 4.7 and 4.8), it can be seen that there has been a slight downward shift of the endotherm when measured on the cooked sample. The size of the amylose-lipid endotherms obtained during storage trial II was larger than that obtained during trial II gelatinization. It has been shown previously that the amylose-lipid complex transition of maize starch was completely reversible (Bulpin *et al.*, 1982). In that study, the complex was found to reform exothermically around 76°C during cooling after the melting process. In the present study, "cooking" scans involved

Table 4.10 Characteristics of the amylose-lipid endotherm for storage trials I and II.

	Temperature (°C)[1]			Heat of transition (J/g CHO)
Batter	T_o	T_p	T_c	
Trial I				
Control	87.4±1.2	102.7±0.5	111.3±1.5	2.7±0.6
SSL	88.1±1.2	103.0±0.5	110.9±0.9	2.5±0.5
MG	89.4±0.9	103.2±0.6	111.1±1.1	2.2±0.5
Trial II				
Control	87.2±1.0	103.0±0.5	111.3±0.7	2.7±0.4
SSL	87.5±0.7	102.8±0.4	110.0±0.7	3.9±0.7
MG	88.8±1.2	103.4±0.2	111.4±0.7	3.1±0.6

[1] Mean±S.D.

heating to only 85°C, so avoiding melting of the complex. The cooked sample had to cool prior to storage thus involving passage through the complex formation temperature range for the second time. During this process it seems likely that additional complexation was able to take place thus resulting in the larger endotherms obtained during analysis of the stored samples. As remarked previously, length of storage did not affect the size of the amylose-lipid endotherm and so cannot be used to explain this phenomenon. This second exposure to complex formation temperatures apparently effected a slight downward temperature shift of the endotherm.

Analysis of variance of amylose-lipid complex data for storage trials I and II showed addition of emulsifiers to have a significant effect on the size of the endotherm. When the Student-Newman-Keuls' (SNK) test with $\alpha = 0.05$ was used to compare the means in storage trial I, it was found that complexation was less in the MG samples than that of the control and SSL which were considered equal. Means comparison for storage trial II revealed that the three means were significantly different from each other (SNK; $\alpha = 0.05$). Complexation decreased in the following order: SSL>MG>control. It may be concluded then, that in trial I, emulsifiers did not complex with the starch and thus did not increase the amount of complex over that occurring in the control. The relative order of amount of complexation found in storage trial II matched that found previously during the gelatinization scans of model batter trial II (Table 4.7).

Staling endotherm data from storage trials I and II are given in Appendix III, Tables 9.1 to 9.6. The data obtained were fitted to the Avrami equation as described in the Experimental section 3.5.4.1. Results of the computer fit are shown in Tables 4.11 and 4.12. Mean values of the endotherm data and the Avrami models were plotted and are presented in Figure 4.13 for trial I and Figure 4.14 for trial II.

Avrami kinetics have been used previously to describe changes occurring during storage of starch gels and bread, the first application being made by Cornford *et al.* (1964) who analysed changes in the firmness of bread crumb. They claimed the Avrami exponent, n, to be 1 and noted that the limiting firmness of the models obtained was not affected by temperature of storage between -1 and 32°C. However, they found that the rate constant, k, decreased as storage temperature was increased, thus confirming the negative temperature coefficient of firming of the crumb. Studies on wheat starch gels using DTA (Colwell *et al.*, 1969) and firmness measurements (Kim and D'Appolonia, 1977a) also resulted in a value of 1 for n. Thus, it was concluded early on that firmness development in crumb and starch gels was caused by crystallization of starch which proceeds by instantaneous nucleation followed by rod-like crystal growth (see Section 3.5.4.1). Since Colwell *et al.* (1969) found a close relationship to exist between the DTA results of wheat

Table 4.11 Results of the Avrami analysis of the staling endotherm during storage trial I.

Sample	Storage temp. (°C)	Limiting heat of transition, ΔH_L (J/g CHO)	95%[1] C.L.	Rate constant, k (days^{-n})	95% C.L.	Avrami exponent, n	95% C.L.	R^2	"F"[2]
Control	5	3.63	±0.83	0.53	±0.21	0.91	±0.62	0.994[3]	0.0
SSL	5	5.39	±1.09	0.28	±0.06	1.05	±0.19	0.993	0.0
MG	5	4.13	±0.56	0.48	±0.09	1.21	±0.28	0.995	0.0
Control	5	3.55	±0.28	0.52	±0.18	1*	-----	0.994	0.04[4]
SSL	5	5.65	±0.74	0.27	±0.06	1*	-----	0.998	0.13[4]
MG	5	4.61	±0.57	0.42	±0.09	1*	-----	0.994	1.42[4]
Control	25	0.58	±0.09	0.013	±0.004	4‡	-----	0.963	0.20[5]
SSL	25	0.74	±0.14	0.010	±0.004	4‡	-----	0.988	0.12[5]
MG	25	0.51	±0.07	0.012	±0.004	4‡	-----	0.988	0.23[5]

[1] 95% confidence limits.
[2] Indication of model appropriateness.
[3] Fraction of variation of data explained by model.
[4] d.f. for "F"; num,den:2,11.
[5] d.f. for "F"; num,den:2,12.

* n set to 1.
‡ Upper boundary condition.

Table 4.12 Results of the Avrami analysis of the staling endotherm during storage trial II.

Sample	Storage temp. (°C)	Limiting heat of transition, ΔH_L (J/g CHO)	95%[1] C.L.	Rate constant, k (days^{-n})	95% C.L.	Avrami exponent, n	95% C.L.	R^2	"F"[2]
Control	5	4.31	±0.81	0.39	±0.09	1.09	±0.29	0.989[3]	0.0
SSL	5	6.65	±8.18	0.21	±0.26	0.89	±0.48	0.987	0.0
MG	5	4.62	±2.18	0.22	±0.11	1.24	±0.52	0.987	0.0
Control	5	4.59	±0.59	0.37	±0.08	1*	-----	0.989	0.23[4]
SSL	5	5.47	±1.25	0.25	±0.10	1*	-----	0.987	0.12[4]
MG	5	7.17	±2.27	0.14	±0.06	1*	-----	0.986	0.35[4]
Control	25	no convergence							
SSL	25	no convergence							
MG	25	0.67	±0.15	0.003	±0.004	4‡	-----	0.929	0.001[4]

[1] 95% confidence limits.
[2] Indication of model appropriateness.
[3] Fraction of variation of data explained by model.
[4] d.f. for "F"; num,den:2,13.

* n set to 1.
‡ Upper boundary condition.

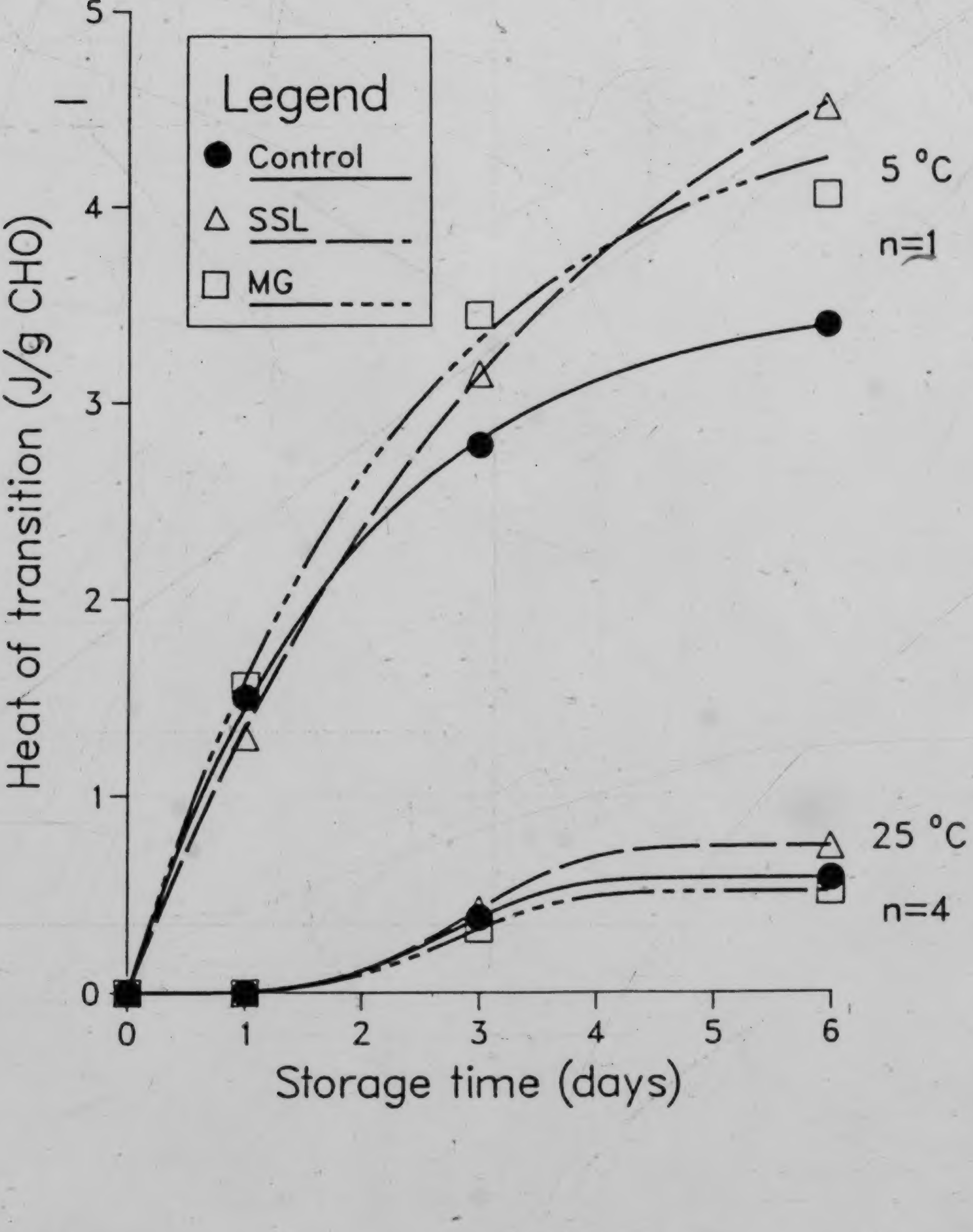


Figure 4.13 Staling endotherm data of storage trial I. Avrami models: n=1 at 5°C storage; n=4 at 25°C storage.

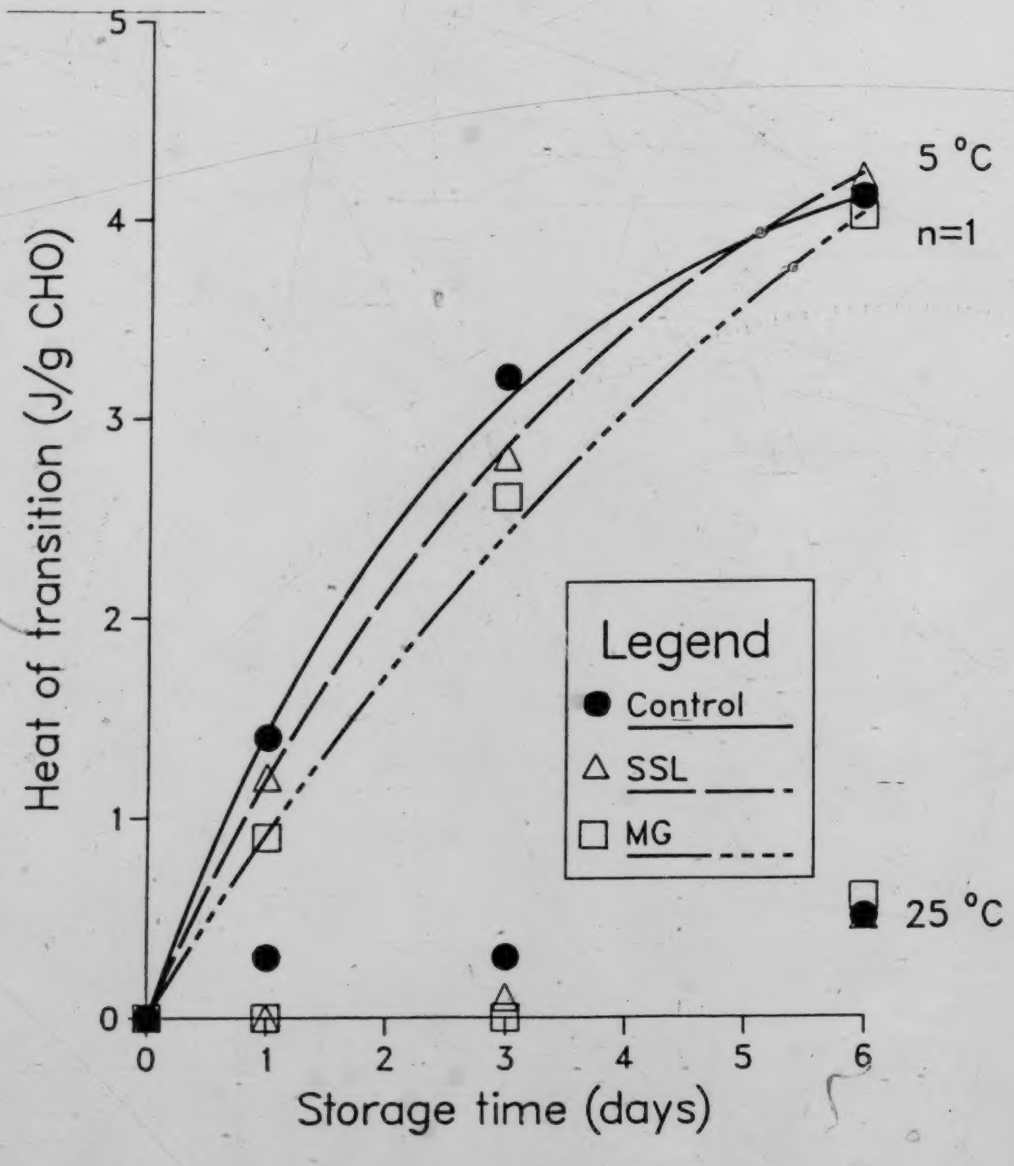


Figure 4.14 Staling endotherm data of storage trial II. Avrami model, n=1 at 5°C.

starch gels and firmness measurements on bread at storage temperatures of -1, 10 and 21°C but not at 32 and 43°C they implicated an unidentified effect playing a role in firming at higher temperatures.

More recently, work has been focussed on the use of the Avrami analysis to characterise starch crystallization and firming during storage in order to quantitate the process and thus to be able to measure the effect on it of various treatments. This was also the motive for the application of the Avrami analysis during the present study. Although the early workers in this area claimed the Avrami exponent, n, to be unity, in the one case where confidence intervals were published (Colwell *et al.*, 1969) these were large enough not to preclude values for n of 0.3 to 2.0. Lately, estimation of the unknown parameters of the Avrami equation has been carried out using computers (Longton and LeGrys, 1981; Fearn and Russell, 1982; Russell, 1983a,b,c) and the issue of a non-integral value for n has been addressed. Longton and LeGrys (1981) used DSC to study crystallization in starch gels and, using R^2 as a measure of fit of the model, found better fits with values for n of 0.4-0.8 than 1. Fearn and Russell (1982) found that analysis of DSC data obtained from bread crumb generated an Avrami model with $n<1$ while good fits to compression data resulted when $n=1$.

It can be seen that for the short-term storage trials of the present study data at 5°C appear to be well fitted by a model with $n=1$. However, the confidence limits for ΔH_L and k for 5°C storage of trial II are of considerable size, indicating that these parameters could have any value within these broad ranges resulting in a model with as good a fit. Confidence intervals for the parameters found for trial I data are smaller. As has been noted previously (Russell, 1983c), since the units of k are dependent on n, it is not valid to compare values of rate constants obtained from models with different values for n.

During parameter estimation for trial I, 25°C storage, models for each treatment converged with n equal to 4, the upper boundary condition. This implies an initial lag period before onset of starch crystallization. In trial I staling endotherms became apparent for all three treatments on day 3 during storage at 25°C. In trial II, control samples first showed a small endotherm on day 1, SSL on day 3 and MG on day 6. Avrami models were not plotted for trial II, 25°C data due to lack of convergence.

Comparison of parameters obtained for storage trial I at 5°C revealed that ΔH_L for SSL and MG samples was greater than that for the control. This would indicate that emulsifiers actually tended to increase the amount of starch crystallization achieved at infinity while SSL but not MG slowed down the rate of crystallization, as is evident by a smaller rate constant. For the 25°C case, rate constants were essentially equal, regardless of emulsifier addition. ΔH_L for SSL was greater than ΔH for MG while neither ΔH_L for SSL nor MG could be differentiated from ΔH_L for control.

It was found that ΔH_L for all cooked batters stored at 25°C was much less than ΔH_L at 5°C and also that the rate constants at 25°C were less than those at 5°C. This is indicative of a greater amount of starch crystallization occurring after long-term storage at 5°C than at 25°C and of a faster rate of crystallization at the lower temperature of storage.

Avrami models for storage trial II at 5°C did not explain as much of the variation of the data as did the models for trial I as indicated by the smaller R^2 values. The confidence intervals for ΔH_L were so large that this parameter as estimated for control, SSL and MG could not be differentiated. Comparison of the the rate constants obtained for Avrami models for 5°C with n equal to unity showed that k for MG was less than k for control while k for SSL could not be differentiated from k for control or k for MG.

The effect of emulsifiers of retarding or reducing staling is largely considered to be due to their starch complexation properties. In trial I, addition of MG and SSL in powder form brought about no extra complexation of amylose and lipid. Therefore, it would be expected that no change in the staling endotherm would result. That the fitted Avrami model should involve MG- and SSL-containing cooked batters having higher values for ΔH_L than the control was unexpected. However, there was some indication of batter containing SSL having a smaller rate constant implying that the rate of starch crystallization was actually reduced.

Data obtained for the samples stored at 25°C during storage trial II were, in general, not well explained by an Avrami model. The size of the staling endotherms was very small throughout storage at 25°C; they were detected in the control cooked batter before the samples containing emulsifiers. Therefore, it appeared that addition of emulsifiers in dispersion form delayed the onset of starch crystallization. This phenomenon may be explained by the fact that MG and SSL addition in trial II was found to increase the size of the amylose-lipid endotherm (Table 4.7).

4.5.2 Long-term storage

4.5.2.1 Storage trial III

X-ray diffraction patterns obtained from crumpets during long-term storage trial III are shown in Figures 4.15 to 4.20. Equivalent d-spacings in Å of the peaks and their areas are given in Tables 4.13 to 4.15. All the diffractograms show the amorphous background typical of gelatinized starch with several peaks superimposed. It can be seen from the results that there are a small number of peaks recurring in many of the diffractograms. These peaks are at approximately 3.7 Å ($2\theta \equiv 28°$), 3.9 Å ($2\theta \equiv 27°$), 5.2 Å ($2\theta \equiv 19.8°$), and 15.2 Å ($2\theta \equiv 6.7°$). Peaks at these four d-spacings are characteristic of the B-pattern of retrograded starch (Zobel, 1964). A fifth peak at around 4.4

Table 4.13 X-ray diffraction patterns of control crumpet.

Length of storage (days)											
1			10			16			30		
d(Å)[1]	2θ (°)[2]	Peak area (cm²)	d(Å)	2θ (°)	Peak area (cm²)	d(Å)	2θ (°)	Peak area (cm²)	d(Å)	2θ (°)	Peak area (cm²)
Storage at 5°C											
	*		3.98	25.97	0.8	3.69	28.06	1.6	3.60	28.77	0.4
			4.44	23.24	1.3	4.02	25.71	0.7	4.03	25.65	1.1
			5.15	20.00	1.3	4.47	23.09	1.6	4.43	23.30	1.5
			14.57	7.04	0.6	5.17	19.93	2.6	5.15	20.00	2.6
						7.82	12.14	0.4	15.15	6.77	1.0
						15.71	6.53	1.1			
Storage at 25°C											
	*			*		4.42	23.35	0.9	4.39	23.51	1.9
						5.11	20.16	1.2	5.10	20.20	1.2
						14.97	6.85	0.5	15.08	6.80	0.9

[1] d, interplanar spacing.
[2] 2θ, diffraction angle.
* no measurable peaks.

Table 4.14 X-ray diffraction patterns of SSL crumpet.

Length of storage (days)											
1			10			16			30		
d(Å)[1]	2θ (°)[2]	Peak area (cm²)	d(Å)	2θ (°)	Peak area (cm²)	d(Å)	2θ (°)	Peak area (cm²)	d(Å)	2θ (°)	Peak area (cm²)
Storage at 5°C											
4.47	23.09	2.6	3.98	25.97	1.4	3.68	28.13	1.9	3.70	27.98	1.7
5.06	20.36	1.0	4.49	22.98	2.3	4.01	25.78	1.2	4.02	25.71	1.6
			5.16	19.96	1.7	4.49	22.98	2.6	4.50	22.93	2.4
			15.61	6.57	1.0	5.19	19.85	2.7	5.19	19.85	2.7
						15.63	6.56	0.9	15.63	6.56	0.9
Storage at 25°C											
4.45	23.09	2.6	5.07	20.32	1.0	4.02	25.71	0.6	5.15	20.18	0.9
5.06	20.36	1.0				4.43	23.30	2.5			
						5.10	20.20	1.5			

[1] d, interplanar spacing.
[2] 2θ, diffraction angle.
* no measurable peaks.

Table 4.15 X-ray diffraction patterns of MG crumpet.

Length of storage (days)											
1			10			16			30		
d(Å)[1]	2θ (°)[2]	Peak area (cm^2)	d(Å)	2θ (°)	Peak area (cm^2)	d(Å)	2θ (°)	Peak area (cm^2)	d(Å)	2θ (°)	Peak area (cm^2)
Storage at 5°C											
	*		3.68	28.13	1.0	4.44	23.24	1.2	3.96	26.11	0.5
			3.98	25.97	0.6	5.10	20.20	0.9	4.39	23.51	0.7
			4.46	23.14	0.8	14.97	6.85	0.7	5.10	20.20	1.1
			5.13	20.08	2.3						
			6.08	16.92	0.6						
			15.38	6.67	0.6						
Storage at 25°C											
	*		4.54	22.72	1.1	4.00	25.84	0.3	4.53	22.78	1.8
			5.18	19.89	1.0	4.45	23.19	1.5	5.08	20.28	1.6
			15.04	6.82	0.3	5.14	20.04	1.4	14.53	7.06	0.4
						15.17	6.76	0.4			

[1] d, interplanar spacing.
[2] 2θ, diffraction angle.
* no measurable peaks.

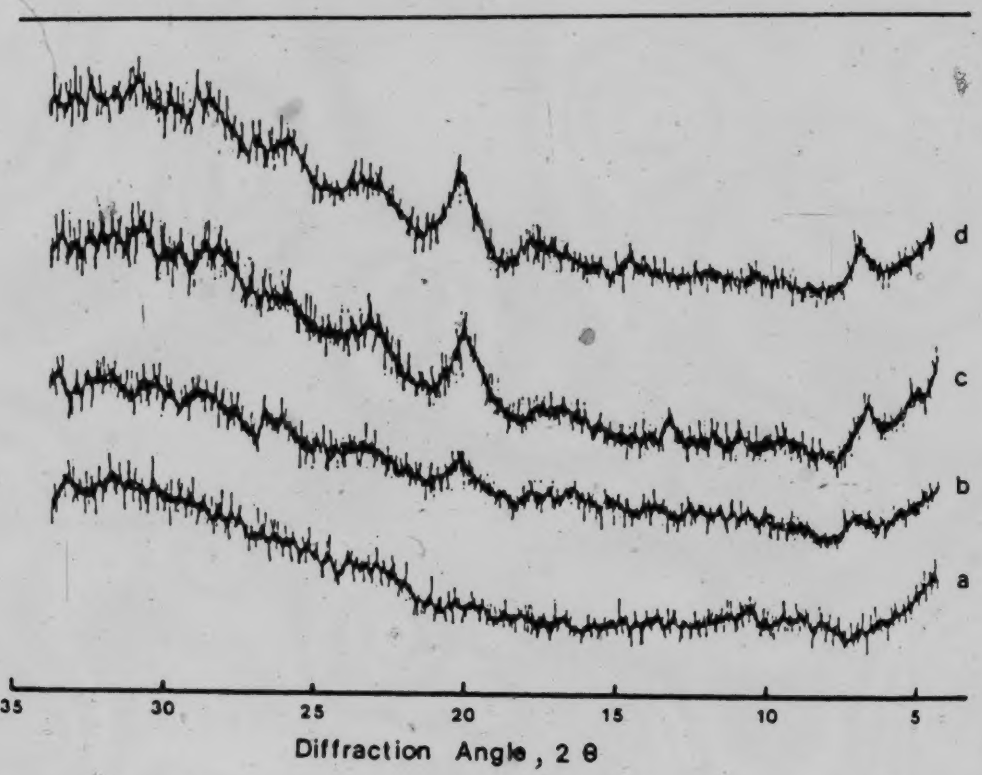


Figure 4.15 X-ray diffraction pattern of control crumpet after storage at 5°C for a, 1; b, 10; c, 16; d, 30 days.

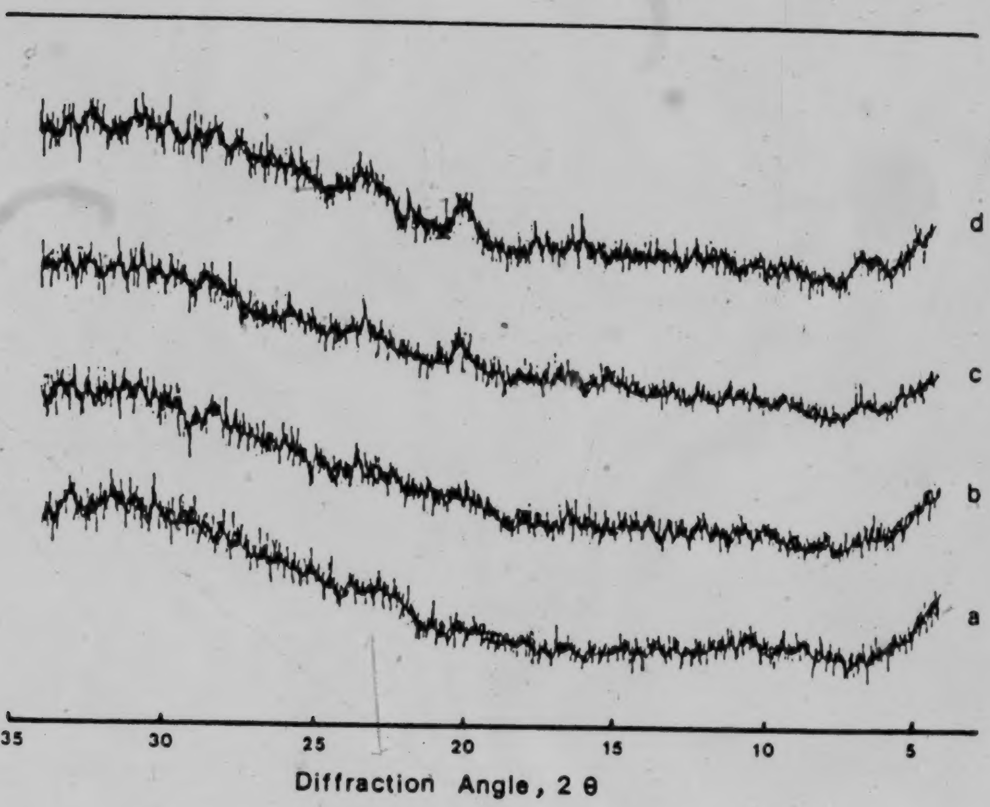


Figure 4.16 X-ray diffraction pattern of control crumpet after storage at 25°C for a, 1; b, 10; c, 16; d, 30 days.

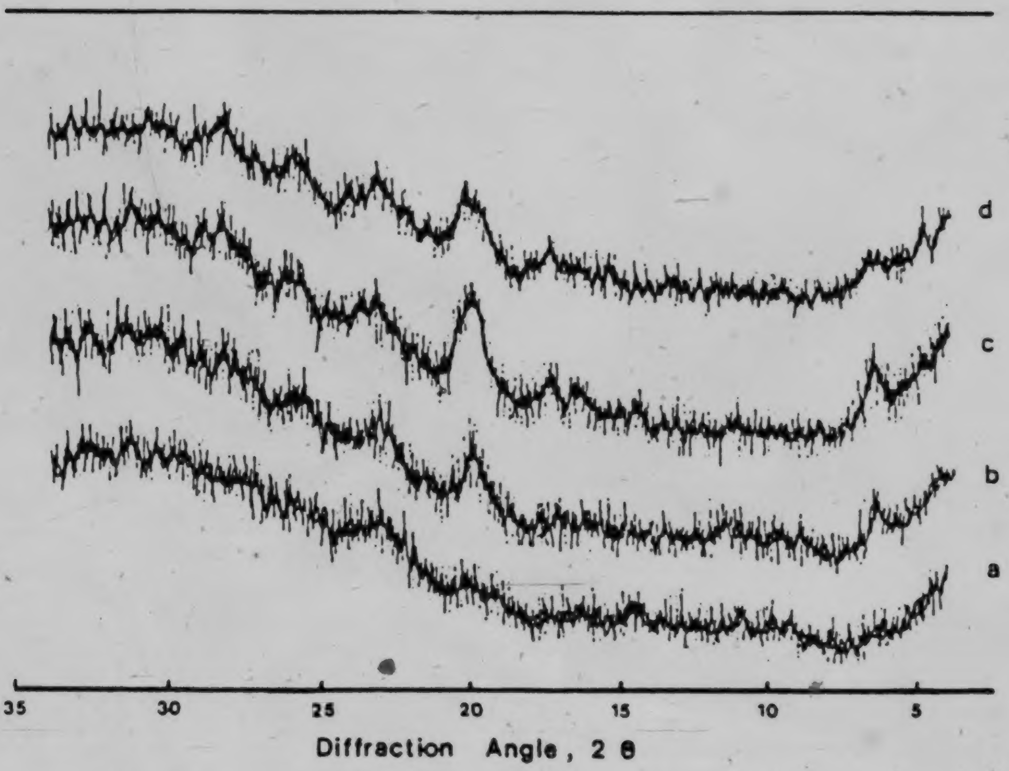


Figure 4.17 X-ray diffraction pattern of SSL crumpet after storage at 5°C for a, 1; b, 10; c, 16; d, 30 days.

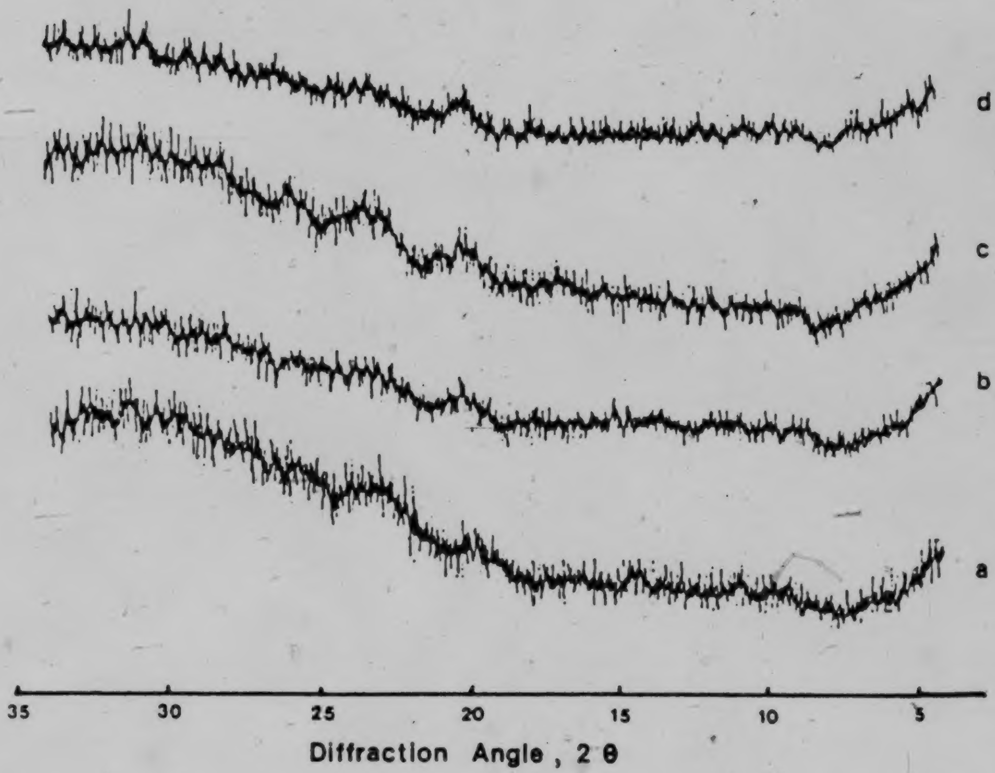


Figure 4.18 X-ray diffraction pattern of SSL crumpet after storage at 25°C for a, 1; b, 10; c, 16; d, 30 days.

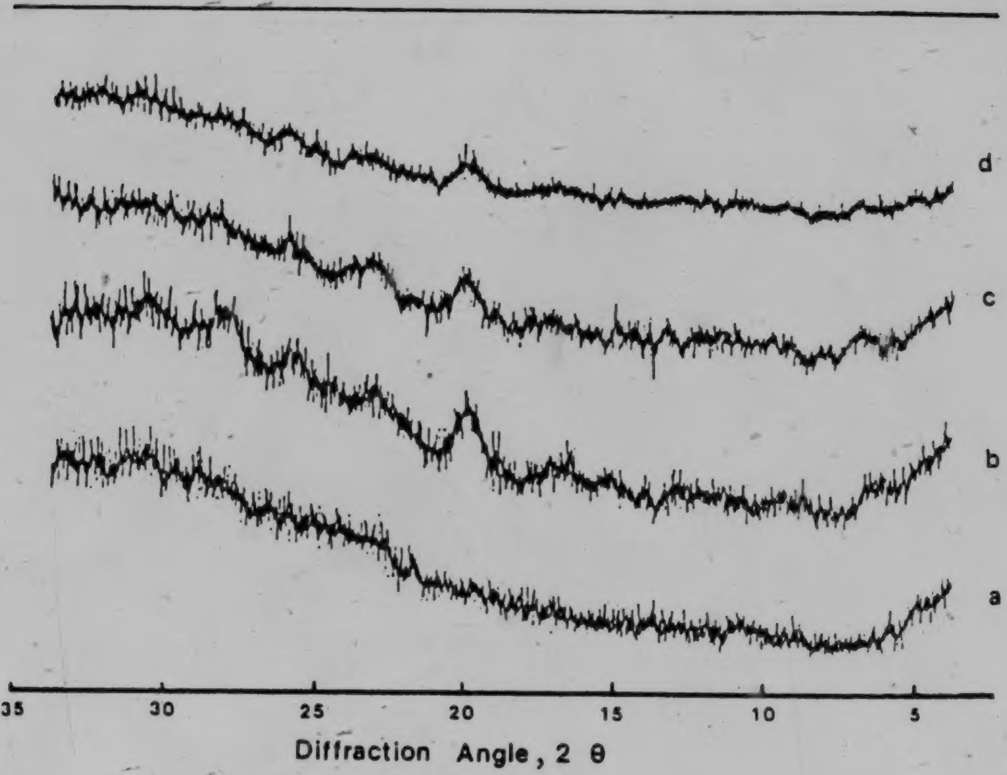


Figure 4.19 X-ray diffraction pattern of MG crumpet after storage at 5°C for a, 1; b, 10; c, 16; d, 30 days.

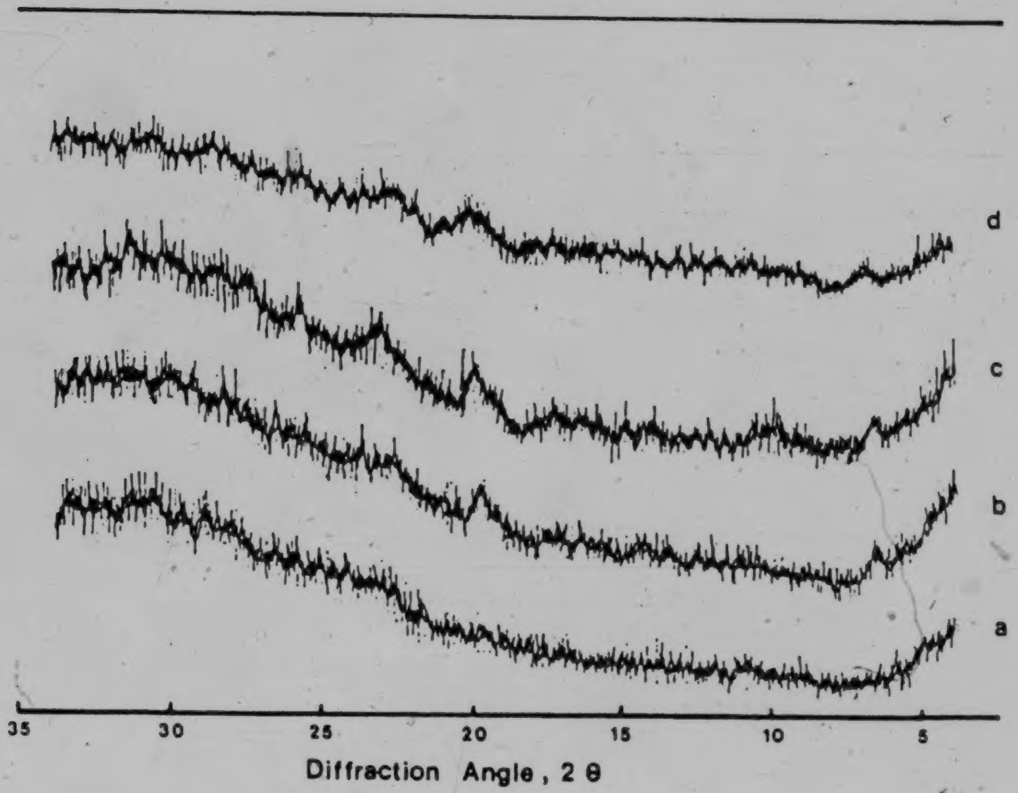


Figure 4.20 X-ray diffraction pattern of MG crumpet after storage at 25°C for a, 1; b, 10; c, 16; d, 30 days.

Å ($2\theta \equiv 23.5°$) also occurred frequently throughout the study. This peak is typical of the V-pattern which arises from complexation between amylose and organic molecules. In the present study, the V-pattern is present due to the amylose-lipid complex. Zobel (1964) noted that small deviations from published characteristic spacings and intensities may be observed due to plant origin of the starch, sample history or moisture content.

The SSL-containing crumpets stored at 5°C (Figure 4.17, Table 4.14) showed a relatively intense peak at 4.4-5.0 Å throughout the storage period. The peak was observed after storage at 25°C for 16 days but not for 10 or 30 days. It would appear then, that SSL addition to the crumpet batter had increased the amount of amylose-lipid complex occurring over that in the MG or control crumpets.

The peak at 5.2 Å has been used to follow crystallization of starch during ageing (Zobel, 1973; Pisesookbunterng *et al.*, 1983). Overall, the peak at 5.2 Å was found to be the most intense of all the peaks observed throughout the present study. This peak was usually more intense for samples stored at 5°C than samples stored at 25°C indicating a greater amount of B-structure or retrograded starch and thus a higher level of crystallinity. This finding concurs with that of Pisesookbunterng *et al.* (1983) that the 5.2 Å peak was more distinct in bread crumb stored at 2°C than that stored at 30°C.

Results given in Figures 4.15 to 4.20 reveal that diffraction patterns of crumpets obtained on day 1 were generally indicative of an amorphous starch structure. Only the SSL-containing crumpets showed measurable peaks on day 1, being a relatively intense peak at 4.4 Å and another at 5.1 Å indicating presence of retrograded starch. In some of the diffraction patterns obtained during the present study the intensity of the 5.1 Å peak was less on day 30 than on day 16 which may be due to fluctuations in the moisture content. It has been noted previously that crystallinity of starches is dependent on moisture content (Zobel, 1964; Nara *et al.*, 1978; Dragsdorf and Varriano-Marston, 1980).

If the beneficial effect of emulsifiers of retarding or reducing staling (Ghiasi *et al.*, 1982b) actually proceeds through complexation with amylose and if staling is due to crystallization of starch, it might be expected that, if there were X-ray evidence of complexation, then the intensity of the B-pattern would be reduced. Even though it was observed in the present study that SSL addition to the crumpet formulation promoted a more intense V-pattern than that observed with control and MG crumpets, there was no evidence of a reduction of the 5.2 Å peak. In fact, the 5.2 Å peak of SSL-crumpets stored at 5°C for 16 and 30 days was equal in intensity to that of the control crumpets stored under equivalent conditions. Pisesookbunterng *et al.* (1983) found that the 5.2 Å peak essentially disappeared after refreshening bread stored at 2°C by heating at 90°C for 45 min while bread stored at 30°C and refreshened still displayed a small peak at 5.2 Å. They concluded that changes other than retrogradation were involved in staling at higher temperatures. Therefore, an additional statement which can be made is that complexation of emulsifier with starch as observed by X-ray diffraction does not necessarily reduce X-ray-determined starch retrogradation.

DSC thermograms obtained during long-term storage trial III were of the typical form as shown previously in Figure 4.12. In some of the thermograms of crumpets containing emulsifiers immeasurably small endotherms were observed centred at the temperatures determined for transition of that particular emulsifier in the presence of water (Table 4.5). This is indicative of the presence of emulsifier free of the amylose-lipid complex.

It was noted that the temperature at which the staling endotherm occurred was dependent on storage temperature. Figure 4.21 shows T_p of the staling endotherm after storage at 5 and 25°C plotted as a function of time. It can be seen that T_p after storage at 25°C was higher than after storage at 5°C. It was also found that the temperature range of the endotherm was narrower after storage at 25°C (26 C°) than after storage at 5°C (32 C°). The characteristic T_p for the staling endotherm was achieved by about the twelfth day of storage after which it remained essentially constant. Addition of emulsifier was not found to affect the characteristic T_p at either storage temperature.

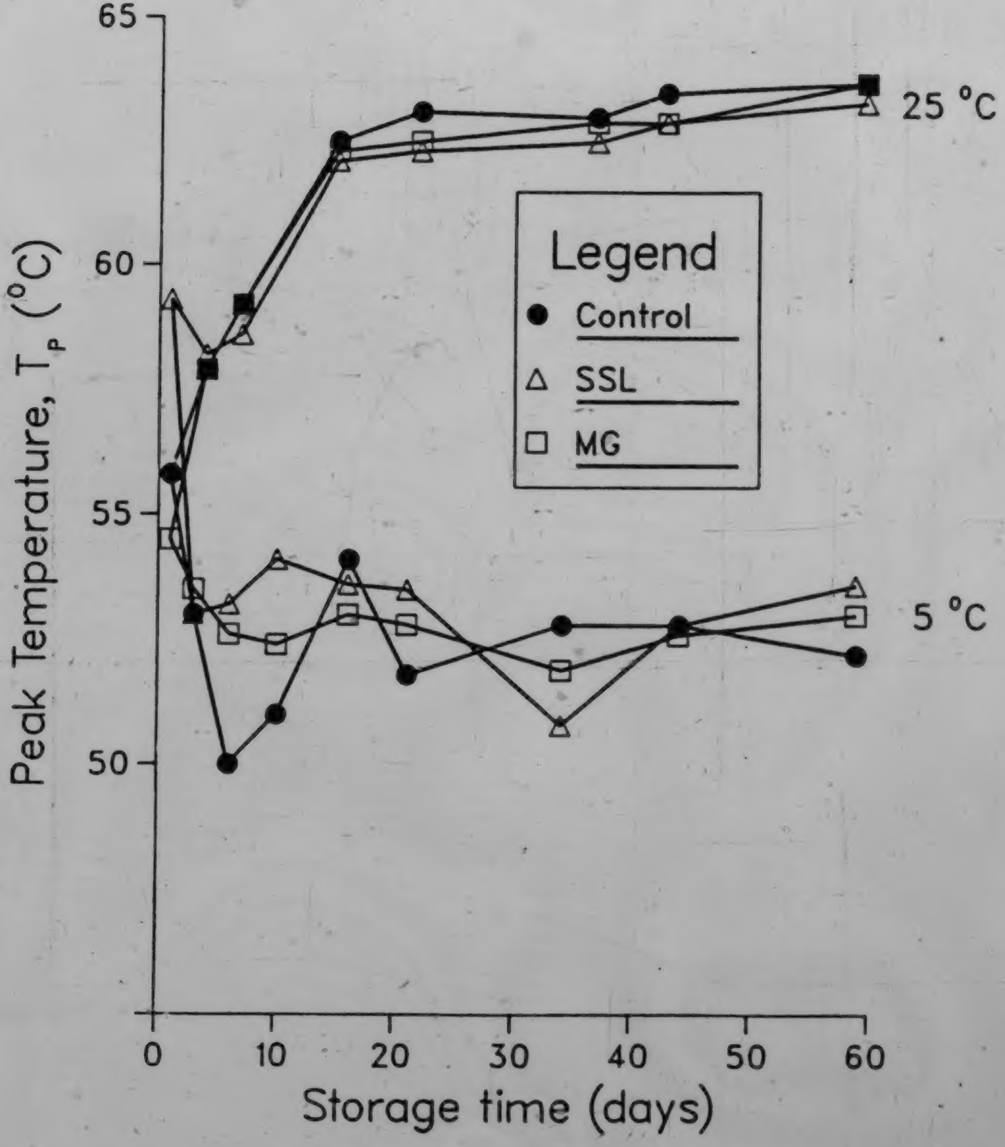


Figure 4.21 **Peak temperature of staling endotherm during storage at 5 and 25°C.**

Differential thermal analysis (DTA) studies on clays have shown that poorly crystalline samples yielded broader endotherms with lower T_P's than well-crystallized samples (Smothers and Chiang, 1966). An upward temperature shift in the DTA peak maximum after storage of starch gels at elevated temperatures was observed by Colwell *et al.* (1969) and after comparison with polymer crystallization (Mandelkern, 1964) was attributed to the formation of a more symmetrically perfect crystal structure. Colwell *et al.* (1969) noted the fact that some degree of supercooling of a polymer below its crystallization temperature is necessary before any measurable crystal formation takes place; the greater the degree of supercooling, the more rapidly the crystals form but the less symmetrically perfect are the resultant crystals. Longton and LeGrys (1981) used DSC to study starch gels after storage at different temperatures and observed that the staling endotherm of gels stored at higher temperatures narrowed and shifted to higher temperatures.

The size of the amylose-lipid complex was followed during storage. As was found during the two short-term trials, length and temperature of storage did not affect the endotherm, so statistics were calculated using all the available data. Results are given in Table 4.16 in J/g crumpet and also converted to J/g CHO. Analysis of variance showed addition of emulsifiers to have a significant effect on endotherm size; means comparison (SNK, α=0.05) revealed

Table 4.16 Size of the amylose-lipid endotherm for storage trial III.

Crumpet batch	Heat of transition[1] (J/g crumpet)	(J/g CHO)
Control	0.48±0.09	1.58±0.29
SSL	0.65±0.16	2.15±0.53
MG	0.52±0.10	1.72±0.32

[1] Mean±S.D.

that addition of SSL at 0.3% of flour weight in powder form resulted in a larger endotherm than that of the control or MG crumpets which are considered equal.

Staling endotherm data obtained during long-term storage trial III are presented in Figure 4.22 in terms of J/g crumpet; experimental means and Avrami models with n=1 have been plotted. The heat of transition of the staling endotherm obtained after 60 days storage at 25°C (ca. 2 J/g crumpet ≅ 6.6 J/g CHO) is slightly less than that reported by Eliasson (1983b) for starch-water gels after 43 days storage at 21°C (9.3 J/g starch). This is probably due to the different storage temperature and the presence of other components in the samples of the present study. Results of the Avrami analysis of the development of the staling endotherm are given in Table 4.17. Avrami analysis of data obtained during storage at 5°C, with the Avrami exponent, n, allowed to vary gave models with n close to unity for all treatments. The fraction of the variation of the data explained by the models was high as indicated by the R^2 values. However, "F" statistics were fairly high, revealing some lack of fit of the models. When a value of 1 was assumed for n, R^2 values were still high while "F" values were slightly increased. Comparison of the estimated parameters showed that ΔH_L for each of the three treatments were equal, a similar condition existing for k. Thus, it appears that addition of emulsifiers in powder form to the crumpet batter at these levels had no effect on the

Table 4.17 Results of the Avrami analysis of the staling endotherm during storage trial III.

Sample	Storage temp. (°C)	Limiting heat of transition, ΔH_L (J/g crumpet)	95%[1] C.L.	Rate constant, k (days^{-n})	95% C.L.	Avrami exponent, n	95% C.L.	R^2	"F"[2]	Degrees of freedom for "F" (num,den)
Control	5	2.28	±0.13	0.13	±0.03	1.18	±0.16	0.977[3]	5.75	6,20
SSL	5	2.38	±0.06	0.13	±0.04	1.15	±0.19	0.982	4.06	6,20
MG	5	2.45	±0.05	0.18	±0.04	0.89	±0.13	0.981	4.98	6,18
Control	5	2.35	±0.14	0.15	±0.02	1*	-----	0.972	6.48	7,20
SSL	5	2.39	±0.06	0.16	±0.03	1*	-----	0.979	4.38	7,20
MG	5	2.42	±0.03	0.15	±0.02	1*	-----	0.978	5.29	7,18
Control	25	no convergence								
SSL	25	5.85	±17.31	0.04	±0.11	0.57	±0.23	0.956	4.47	5,17
MG	25	2.19	±0.77	0.07	±0.02	0.78	±0.13	0.978	1.97	5,18
Control	25	1.57	±0.12	0.08	±0.01	1*	-----	0.952	34.68	6,18
SSL	25	1.89	±0.25	0.05	±0.01	1*	-----	0.926	8.23	6,17
MG	25	1.64	±0.16	0.06	±0.01	1*	-----	0.964	4.47	6,18

[1] 95% confidence limits.

[2] Indication of model appropriateness.

[3] Fraction of variation of data explained by model.

* n set to 1.

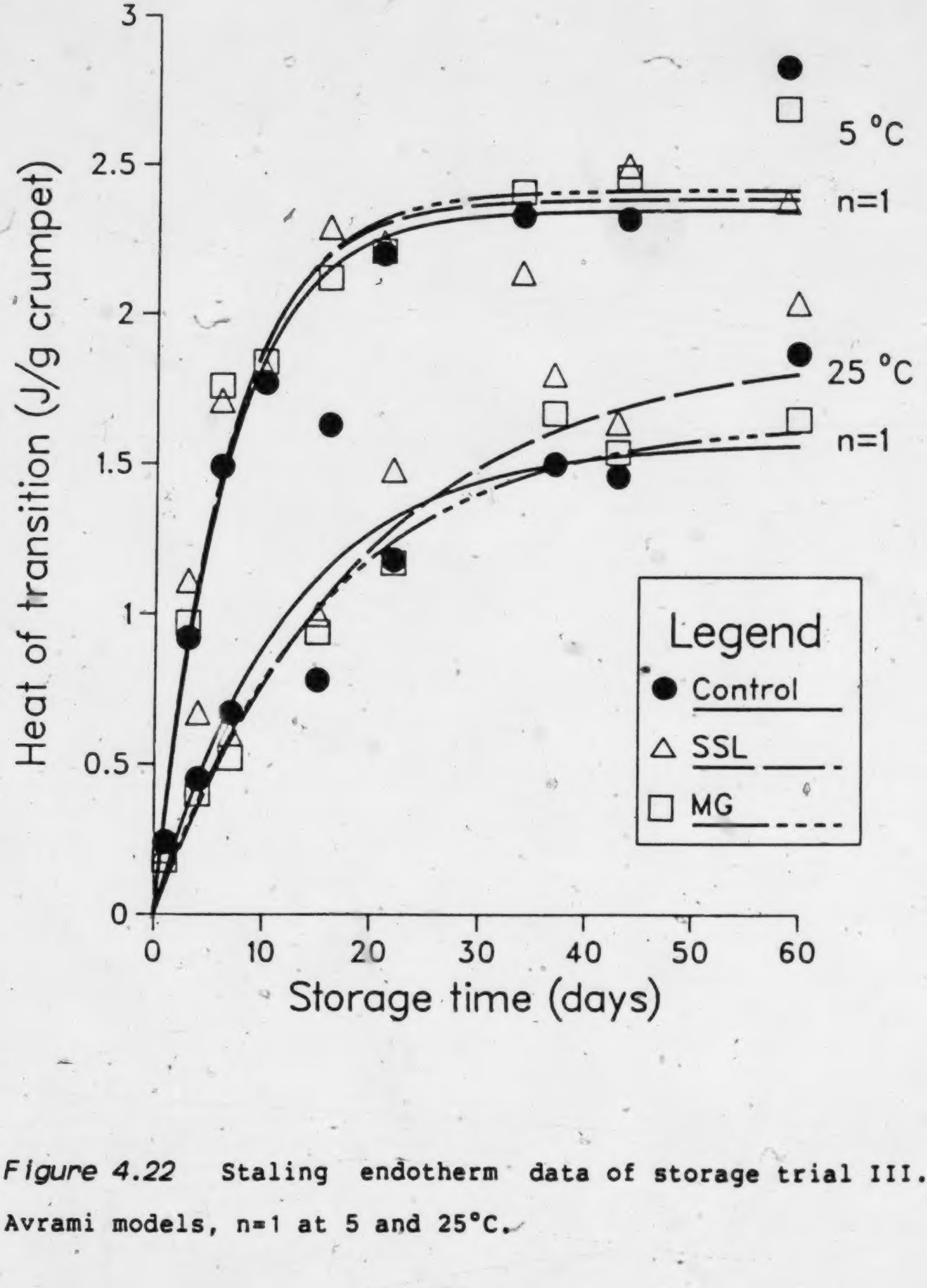


Figure 4.22 Staling endotherm data of storage trial III. Avrami models, n=1 at 5 and 25°C.

crystallization of starch as measured using DSC during storage at 5°C. DSC results therefore complement those obtained using X-ray diffraction in that, even though there was evidence that SSL crumpets contained more amylose-lipid complex than did MG or control crumpets, there was no indication of a resultant decrease in starch crystallization.

There was no detection of a lag period before the appearance of a staling endotherm for crumpets stored at 25°C as had been found with short-term storage of cooked batter in DSC pans. However, since the product for the long-term storage trial had had some unavoidable exposure to uncontrolled temperatures during transportation from the factory to the laboratory, it is possible that this hastened the onset of staling.

When the data obtained from 25°C storage of control crumpets were analysed using Avrami kinetics convergence was not achieved. Models for SSL- and MG-containing crumpets converged with $n<1$, but confidence intervals for the parameters were large, especially for SSL. When n was set to unity, convergence was achieved but the "F" statistics were significant, indicating lack of model appropriateness. It appears that crystallization of starch during storage at 25°C of control and SSL-containing crumpets cannot be described by an Avrami model with $n=1$. Data obtained from MG-containing crumpets stored at 25°C fit well to a model with $n=0.78$; model appropriateness was made worse when n was set to 1 but not as bad as for control and SSL crumpets. Since rate constants cannot be compared unless the Avrami exponents of the models are equal, it is not possible to discuss differences or similarities of k values obtained for the samples stored at 25°C. When the model for MG crumpets stored at 25°C with n set to 1 is compared to the equivalent model for 5°C storage, it is apparent that ΔH_L at 25°C is less than ΔH_L at 5°C.

Results obtained from compression testing of crumpets during long-term storage trial III are presented in Table 4.18. It can be seen that crumpets stored at 5°C for any particular length of time were firmer than those stored for the same length of time at 25°C. In some cases data obtained were rather erratic; for example, SSL- and MG-containing crumpets stored at 5°C increased in firmness to day 21, gave a decreased value at day 34, then increased to the end of the trial. At both storage temperatures, SSL and MG crumpets were firmer than control crumpets.

Results of the Avrami analysis of the firmness of crumpets during storage trial III are shown in Table 4.19. The fit of the firmness data to an Avrami model was generally worse than that obtained with staling endotherm data. This may be due to the fact that the analysis by DSC of the staling endotherm is measuring starch crystallization directly and is unrelated to the macrostructure of the sample. Results from compression methods are dependent on the structure of the sample and the use of Avrami kinetics

Table 4.18 Firmness of crumpets; long-term storage trial III.

Length of storage (days)	Firmness (kgf/mm)[1] Control	SSL	MG
Storage at 5°C			
1[2]	0.14±0.02	0.22±0.01	0.20±0.01
3	0.33±0.02	0.51±0.02	0.51±0.07
6	0.55±0.03	0.82±0.09	0.87±0.05
10	0.81±0.15	1.15±0.08	0.92±0.12
16	0.91±0.13	1.16±0.05	1.24±0.09
21	1.15±0.29	1.82±0.07	1.71±0.25
34	1.20±0.20	1.58±0.20	1.42±0.19
44	1.15±0.18	2.30±0.20	1.97±0.15
59	1.46±0.21	2.64±0.22	2.10±0.20
Storage at 25°C			
1[2]	0.14±0.02	0.22±0.01	0.20±0.01
4	0.21±0.01	0.34±0.01	0.30±0.01
7	0.26±0.04	0.38±0.02	0.38±0.01
15	0.36±0.04	0.51±0.04	0.52±0.03
22	0.39±0.01	0.62±0.03	0.58±0.03
37	0.57±0.04	0.85±0.06	0.75±0.06
43	0.56±0.06	0.95±0.04	0.79±0.06
60	0.62±0.03	1.09±0.06	1.01±0.05

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[1] Mean±S.D.
[2] Initial determination before storage.

Table 4.19 Results of the Avrami analysis of firmness of crumpets during storage trial III.

Sample	Storage temp. (°C)	Limiting firmness, F_L (kgf/mm)	95%[1] C.L.	Rate constant, k (days^{-n})	95% C.L.	Avrami exponent, n	95% C.L.	R^2	"F"[2]	Degrees of freedom for "F" (num,den)
Control	5	1.19	±0.15	0.037	±0.013	1.45	±0.23	0.946[3]	4.91	6,26
SSL	5	2.22	±0.69	0.018	±0.011	1.38	±0.24	0.907	87.90	6,27
MG	5	no convergence								
Control	5	1.43	±0.29	0.056	±0.017	1*	-----	0.908	9.89	7,26
SSL	5	5.57	±7.04	0.012	±0.019	1*	-----	0.858	117.43	7,27
MG	5	2.58	±1.07	0.031	±0.020	1*	-----	0.850	51.70	7,27
Control	25	0.88	±0.38	0.024	±0.008	0.93	±0.19	0.970	3.43	5,24
SSL	25	1.44	±0.81	0.020	±0.010	1.01	±0.18	0.963	24.66	5,24
MG	25	1.23	±0.47	0.025	±0.008	0.97	±0.15	0.970	9.21	5,24
Control	25	0.79	±0.13	0.023	±0.007	1*	-----	0.970	3.00	6,24
SSL	25	1.48	±0.44	0.019	±0.009	1*	-----	0.963	20.56	6,24
MG	25	1.15	±0.17	0.026	±0.007	1*	-----	0.970	7.78	6,24

[1] 95% confidence limits.
[2] Indication of model appropriateness.
[3] Fraction of variation of data explained by model.

* n set to 1.

to analyse such data is based on the assumption that changes in firmness reflect changes in starch crystallization. For Avrami analysis of staling endotherm data it is assumed that at time, t=0, there is no starch crystallization, i.e. that $\Delta H_0=0$, whereas with compression data, firmness at t=0 is not equal to zero. Since, in the present study, it was impossible to measure firmness immediately after manufacture, the initial determination made on day 1 (day of manufacture being day 0) was used for F_0.

Values for R^2 obtained for the Avrami models were all high, indicating significant explanation of the variation of the data by the models. However, confidence intervals for many of the estimated parameters were extremely large, implying that the value of the parameters could vary widely. Since "F" values were high, some extremely so, it may be concluded that, generally, Avrami kinetics cannot be used to describe changes in the firmness of crumpets during storage.

Results obtained from compression of the crumpet "cell-wall material" are presented in Table 4.20. To some extent, the results obtained from compression of crumpets, as shown in Table 4.18, are reflected here; "cell-wall material" from SSL- and MG-containing crumpets was firmer than that from control crumpets. This would indicate that such data obtained from crumpet compression was not due to differences in specific volume. Firmness of the "cell-wall material" was greater after storage at 5°C than after storage at 25°C. Avrami analysis of the data resulted in the

Table 4.20 Firmness of crumpet "cell-wall material"; long-term storage trial III.

Length of storage (days)	Firmness (kgf/mm)[1] Control	SSL	MG
Storage at 5°C			
1[2]	0.26±0.02	0.30±0.01	0.29±0.01
3	0.42±0.01	0.54±0.02	0.52±0.04
6	0.57±0.02	0.68±0.01	0.68±0.05
10	0.78±0.08	1.00±0.07	0.93±0.01
16	0.89±0.06	1.00±0.02	1.04±0.04
21	0.81±0.19	1.13±0.04	1.25±0.11
34	1.23±0.09	1.34±0.01	1.29±0.07
44	1.45±0.28	1.87±0.18	1.73±0.02
59	1.66±0.29	1.92±0.22	1.61±0.10
Storage at 25°C			
1[2]	0.26±0.02	0.30±0.01	0.29±0.01
4	0.30±0.01	0.38±0.01	0.40±0.01
7	0.31±0.01	0.45±0.06	0.36±0.03
15	0.39±0.05	0.58±0.06	0.49±0.05
22	0.30±0.05	0.60±0.09	0.70±0.16
37	0.49±0.04	0.88±0.06	0.55±0.10
43	0.40±0.08	0.91±0.19	0.88±0.10
60	0.44±0.13	1.04±0.14	0.70±0.12

[1] Mean±S.D.
[2] Initial determination before storage.

models given in Table 4.21. In most of the cases "F" for lack of fit was highly significant, indicating severe lack of model appropriateness. Thus, a similar conclusion to that relating to compression of crumpets may be arrived at: that firming of crumpet "cell-wall material" is not well-described by Avrami kinetics.

4.5.2.2 Storage trial IV

DSC thermograms obtained during long-term storage trial IV were of the typical form as shown previously in Figure 4.12. Unlike the thermograms obtained during storage trial III, there was no evidence of endotherms due to melting of the emulsifiers indicating that all the SSL added in dispersion form had complexed with the amylose.

The size of the amylose-lipid complex was followed during storage; results are given in Table 4.22. Analysis of variance of the data showed that addition of SSL significantly affected the size of the endotherm. Comparison of means (SNK, $\alpha=0.05$) indicated that the size of the amylose-lipid endotherm of the control crumpets was less than those of the SSL-containing crumpets. However, since the size of the endotherm of crumpets containing 0.3% (of flour weight) SSL was not different from that of crumpets containing 0.45% (of flour weight) SSL, it could not be concluded that a higher level of SSL results in a greater amount of complexation.

Staling endotherm data are presented in Figure 4.23; means of experimental data and Avrami models with $n=1$ have

Table 4.21 Results of the Avrami analysis of firmness of crumpet cell wall material during storage trial III.

Sample	Storage temp. (°C)	Limiting firmness, F_L (kgf/mm)	95%[1] C.L.	Rate constant, k (days^{-n})	95% C.L.	Avrami exponent, n	95% C.L.	R^2	"F"[2]	Degrees of freedom for "F" (num,den)
Control	5	1.32	±0.28	0.045	±0.013	1.14	±0.20	0.945[3]	7.85	6,18
SSL	5	1.31	±0.07	0.024	±0.015	1.64	±0.34	0.980	94.44	7,19
MG	5	1.69	±0.10	0.022	±0.015	1.44	±0.30	0.990	55.71	6,18
Control	5	1.56	±0.36	0.044	±0.015	1*	-----	0.941	7.54	7,18
SSL	5	1.59	±0.27	0.050	±0.018	1*	-----	0.951	238.62	7,19
MG	5	1.89	±0.22	0.050	±0.010	1*	-----	0.980	100.87	7,18
Control	25	no convergence								
SSL	25	0.85	±0.13	0.019	±0.008	1.52	±0.34	0.948	16.33	5,16
MG	25	0.60	±0.14	0.053	±0.047	1.40	±0.66	0.827	18.09	5,16
Control	25	0.52	±0.17	0.032	±0.025	1*	-----	0.703	4.81	6,16
SSL	25	1.77	±1.62	0.013	±0.015	1*	-----	0.906	26.62	6,16
MG	25	0.74	±0.24	0.057	±0.038	1*	-----	0.810	16.83	6,16

[1] 95% confidence limits.
[2] Indication of model appropriateness.
[3] Fraction of variation of data explained by model.

* n set to 1.

Table 4.22 Size of the amylose-lipid endotherm for storage trial IV.

Crumpet batch	Heat of transition[1] (J/g crumpet)	(J/g CHO)
Control	0.73±0.17	2.40±0.55
SSL, 0.3%[2]	1.11±0.20	3.67±0.65
SSL, 0.45%[2]	1.18±0.22	3.88±0.73

[1] Mean±S.D.
[2] Based on flour weight.

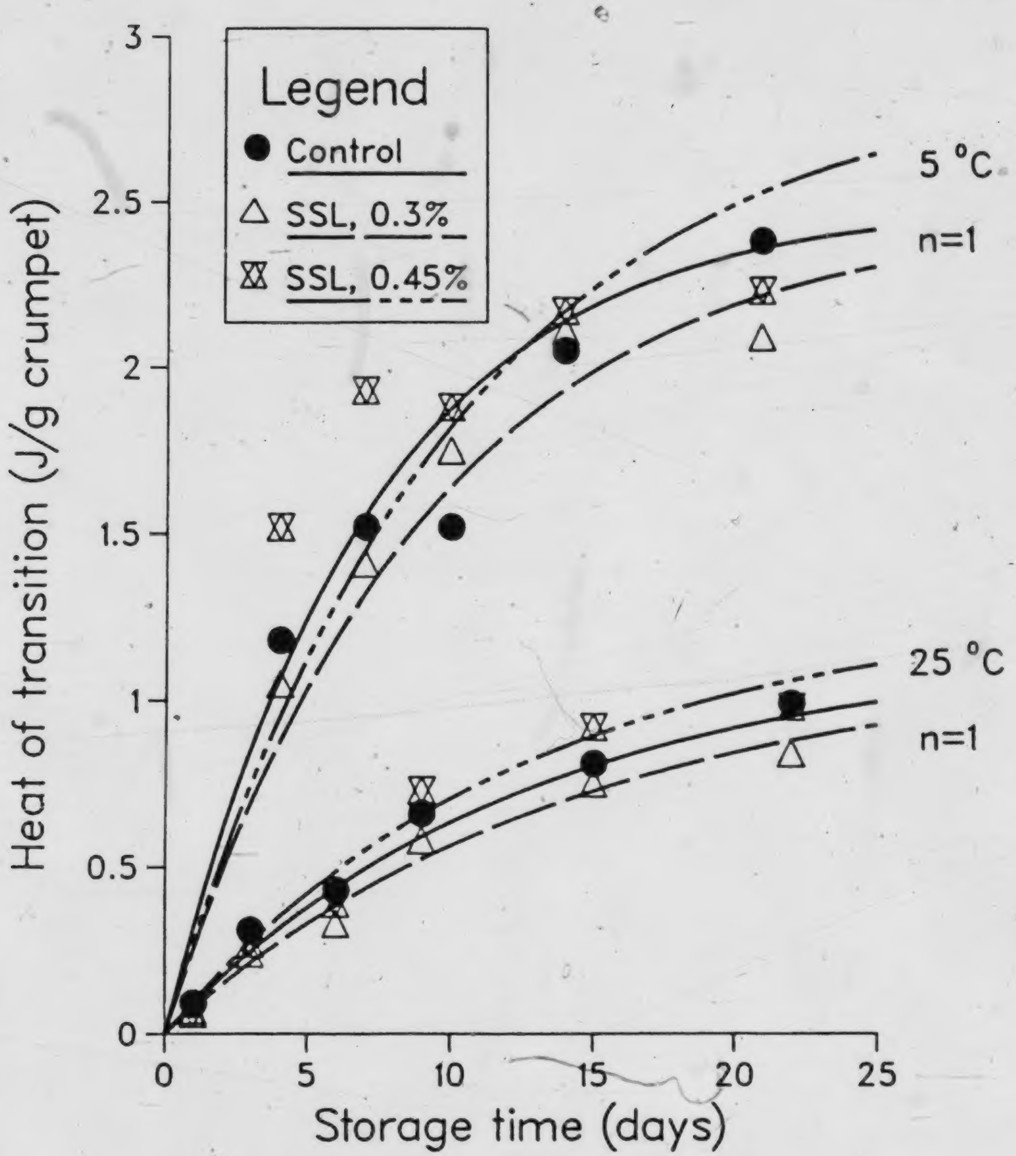

Figure 4.23 Staling endotherm data of storage Trial IV. Avrami models, n=1 at 5 and 25°C.

been plotted. Results of the Avrami analysis of endotherm data are shown in Table 4.23. When Avrami analysis was carried out on the data obtained during 25°C storage with n allowed to vary, no convergence was achieved for control or SSL (0.45%) crumpets. When the data were reanalysed with n set to 1, models were obtained with high R^2 and low "F" values. Comparison of values of ΔH_L and k obtained revealed that addition of SSL at either level had no significant effect on starch crystallization. This therefore reinforces the earlier conclusion that even though emulsifiers may have complexed with amylose they do not necessarily affect the starch crystallization kinetics.

Avrami analysis of 5°C data gave similar problems with convergence as found for the data obtained from 25°C storage. Again, when all parameters were allowed to vary, convergence was achieved only for SSL (0.3%) data. When n was set to 1 the models obtained had very high "F" values indicating severe lack of model appropriateness.

Results obtained from compression testing of crumpets during long-term storage trial IV are presented in Table 4.24. As noted in storage trial III, crumpets stored at 5°C were firmer than those stored at 25°C for the same length of time. After storage at 5°C, control crumpets developed approximately the same firmness as crumpets containing SSL at a level of 0.45% (of flour weight) while 0.3% SSL crumpets were more firm. A similar conclusion may be drawn for the crumpets stored at 25°C.

Table 4.23 Results of the Avrami analysis of the staling endotherm during storage trial IV.

Sample	Storage temp. (°C)	Limiting heat of transition, ΔH_L (J/g crumpet)	95%[1] C.L.	Rate constant, k (days^{-n})	95% C.L.	Avrami exponent, n	95% C.L.	R^2	"F"[2]	Degrees of freedom for "F" (num,den)
Control	5	no convergence								
SSL 0.3%	5	2.05	±0.12	0.06	±0.03	1.72	±0.31	0.982	9.80	3,20
SSL 0.45%	5	no convergence								
Control	5	2.49	±0.18	0.14	±0.03	1*	-----	0.955	30.71	4,22
SSL 0.3%	5	2.46	±0.44	0.11	±0.04	1*	-----	0.942	35.78	4,20
SSL 0.45%	5	2.88	±0.89	0.10	±0.06	1*	-----	0.947	34.22	4,18
Control	25	no convergence								
SSL 0.3%	25	1.00	±0.32	0.06	±0.02	1.10	±0.32	0.958	3.38	3,19
SSL 0.45%	25	no convergence								
Control	25	1.15	±0.29	0.08	±0.03	1*	-----	0.925	1.30	4,19
SSL 0.3%	25	1.12	±0.24	0.07	±0.02	1*	-----	0.957	2.67	4,19
SSL 0.45%	25	1.28	±0.28	0.08	±0.03	1*	-----	0.954	3.69	4,21

[1] 95% confidence limits.
[2] Indication of model appropriateness.
[3] Fraction of variation of data explained by model.

* n set to 1.

Table 4.24 Firmness of crumpets; long-term storage trial IV.

Length of storage (days)	Firmness (kgf/mm)[1] Control	SSL, 0.3%	SSL, 0.45%
Storage at 5°C			
1[2]	0.12±0.01	0.21±0.01	0.16±0.01
4	0.51±0.03	0.75±0.04	0.70±0.07
7	0.76±0.04	1.15±0.13	0.98±0.12
10	0.76±0.10	1.12±0.15	0.88±0.14
14	1.06±0.08	1.45±0.11	0.94±0.16
21	1.29±0.05	1.63±0.04	1.34±0.12
Storage at 25°C			
1[2]	0.12±0.01	0.21±0.01	0.16±0.01
3	0.16±0.01	0.29±0.01	0.23±0.01
6	0.23±0.01	0.38±0.01	0.29±0.06
9	0.29±0.01	0.45±0.02	0.37±0.03
15	0.31±0.06	0.56±0.03	0.42±0.06
22	0.42±0.01	0.66±0.04	0.48±0.03

[1] Mean±S.D.
[2] Initial determination before storage.

Avrami analysis of the firmness data yielded the results given in Table 4.25. Analysis of the data obtained after storage at 5°C resulted in models with high "F" values indicating that changes in firmness at this temperature are not well-described by Avrami kinetics. Models for the 25°C storage runs converged with n approximately equal to 1.5. When the data were reanalysed with n set to 1.5 the R^2 values were unchanged; the "F" statistics for control and 0.45% SSL crumpets were low while that for 0.3% SSL crumpets was rather high. When the estimated parameters of these models were compared it was found that the rate constants were essentially equal whereas the limiting firmness of SSL (0.3%) crumpets was greater than that of the control and SSL (0.45%) crumpets which were approximately equal.

Data obtained from compression of crumpet "cell-wall material" are given in Table 4.26. As expected, firmness after 5°C storage was greater than that after 25°C storage. It is very difficult to distinguish between results obtained for the three treatments after 5°C storage while, after storage at 25°C, control and SSL (0.3%) samples were as firm as each other at any particular storage time. The "cell-wall material" of the SSL (0.45%) crumpets showed no increase in firmness until the fifteenth day of storage.

Results obtained from Avrami analysis of the firmness of the crumpet "cell-wall material" are shown in Table 4.27. Generally, the values obtained for R^2 are high and those for "F" are low. However, the confidence intervals for k are

Table 4.25 Results of the Avrami analysis of firmness of crumpets during storage trial IV.

Sample	Storage temp. (°C)	Limiting firmness, F_L (kgf/mm)	95%[1] C.L.	Rate constant, k (days^{-n})	95% C.L.	Avrami exponent, n	95% C.L.	R^2	"F"[2]	Degrees of freedom for "F" (num,den)
Control	5	1.23	±0.12	0.029	±0.019	1.76	±0.38	0.968[3]	32.36	3,18
SSL 0.3%	5	1.57	±0.08	0.007	±0.009	3.07	±1.02	0.985	28.59	3,18
SSL 0.45%	5	1.06	±0.10	0.004	±0.001	4‡	-----	0.946	7.72	4,18
Control	25	0.44	±0.02	0.028	±0.010	1.52	±0.20	0.995	2.67	3,18
SSL 0.3%	25	0.63	±0.08	0.031	±0.014	1.55	±0.31	0.973	10.91	3,18
SSL 0.45%	25	0.48	±0.04	0.039	±0.019	1.51	±0.32	0.959	1.59	3,18
Control	25	0.44	±0.01	0.029	±0.003	1.5*	-----	0.995	2.03	4,16
SSL 0.3%	25	0.64	±0.07	0.033	±0.008	1.5*	-----	0.973	8.26	4,18
SSL 0.45%	25	0.48	±0.03	0.039	±0.010	1.5*	-----	0.959	1.19	4,18

[1] 95% confidence limits.
[2] Indication of model appropriateness.
[3] Fraction of variation of data explained by model.
‡ upper boundary condition.
* n set to 1.5.

Table 4.26 Firmness of crumpet "cell-wall" material; long-term storage trial IV.

Length of storage (days)	Firmness (kgf/mm)[1] Control	SSL, 0.3%	SSL, 0.45%
Storage at 5°C			
1[2]	0.22±0.02	0.22±0.02	0.24±0.03
4	0.55±0.06	0.45±0.03	0.45±0.05
7	0.58±0.06	0.56±0.07	0.58±0.12
10	0.65±0.01	0.75±0.11	0.57±0.11
14	0.76±0.01	0.68±0.04	0.53±0.08
21	0.88±0.10	0.81±0.11	0.77±0.15
Storage at 25°C			
1[2]	0.22±0.02	0.22±0.02	0.24±0.03
3	0.25±0.04	0.25±0.03	0.21±0.02
6	0.29±0.02	0.27±0.03	0.22±0.01
9	0.31±0.01	0.29±0.04	0.22±0.01
15	0.32±0.02	0.33±0.03	0.25±0.01
22	0.33±0.01	0.32±0.03	0.29±0.04

[1] Mean±S.D.
[2] Initial determination before storage.

Table 4.27 Results of the Avrami analysis of firmness of crumpet cell wall material during storage trial IV.

Sample	Storage temp. (°C)	Limiting firmness, F_L (kgf/mm)	95%[1] C.L.	Rate constant, k (days^{-n})	95% C.L.	Avrami exponent, n	95% C.L.	R^2	"F"[2]	Degrees of freedom for "F" (num,den)
Control	5	0.89	±0.19	0.049	±0.042	1.34	±0.55	0.978[3]	8.99	3,12
SSL 0.3%	5	0.69	±0.05	0.025	±0.043	2.32	±1.23	0.964	3.22	3,12
SSL 0.45%	5	0.58	±0.07	0.004	±0.003	4‡	-----	0.854	1.39	4,12
Control	5	0.85	±0.07	0.038	±0.011	1.5*	-----	0.978	7.08	4,12
SSL 0.3%	5	0.71	±0.07	0.069	±0.024	1.5*	-----	0.949	4.76	4,12
SSL 0.45%	5	0.60	±0.09	0.100	±0.068	1.5*	-----	0.822	2.38	4,12
Control	25	0.33	±0.01	0.037	±0.093	1.83	±1.27	0.853	0.31	3,12
SSL 0.3%	25	0.33	±0.04	0.037	±0.097	1.59	±1.49	0.784	0.35	3,12
SSL 0.45%	25	#								

[1] 95% confidence limits.
[2] Indication of model appropriateness.
[3] Fraction of variation of data explained by model.

‡ upper boundary condition.
* n set to 1.5.
# converged with n=0.

extremely large in the majority of the obtained models, indicating a high degree of uncertainty regarding their value.

When the parameters to be estimated were all allowed to vary, models for the 5°C storage case converged with different n values. When n was set to 1.5, the limiting firmness values obtained decreased in the following order: control > 0.3% SSL > 0.45% SSL.

It was impossible to differentiate between the models obtained for control and SSL (0.3%) samples stored at 25°C. The model obtained for SSL (0.45%) "cell-wall material" comprised an Avrami exponent of zero resulting from the lack of firmness increase with storage time.

It was found in the present study that analysis of DSC results obtained from 5°C storage could often be well described by an Avrami model where n=1, but there were exceptions. It was also found that appropriate models obtained for the 25°C data did not usually include an Avrami exponent equal to 1.

In the present study lower limiting values for heat of transition (ΔH_L) were obtained for Avrami models at 25°C than for those a 5°C storage. This is in agreement with the results obtained by Colwell *et al.* (1969). They suggested that a lower limiting value implies less total crystallization in a totally stale sample stored at high temperature than one stored at low temperature. They concluded that an unidentified temperature dependent feature prevented all the crystallizable material present from crystallizing, since initially samples were in the same compositional state. Results obtained by Longton and LeGrys (1981) indicated that ΔH_L at 4°C tended to be greater than at 21 and 30°C; they also concluded that variation of n between 4 and 30°C was caused by a temperature effect.

As was noted in the present study, crystal form is dependent on temperature. Morgan (1955) proposed that it is reasonable to suppose that there is present in a crystallized polymer a pattern or arrangement of crystalline and amorphous regions and that the particular arrangement is specific to the conditions holding during the crystallization. Since the Avrami exponent relates to crystallite morphology one would expect it to change with storage temperature.

Data obtained during the present study did not show that addition of emulsifiers to crumpet batter had any significant effect on either development of the staling endotherm or on firmness of the product. This is not in agreement with the findings of Russell (1983b) that addition of MG in the gel-state to bread reduced the rate constant determined from DSC data, although it had no effect on ΔH_L. However, Russell used a level of MG of 1% based on flour weight which is considerably more than was used in the present study. Russell (1983b) also used compressibility measurements and found that MG reduced the rate constant of firming but he found no functional relationship between the rate constant determined using DSC data and that determined using compressibility data.

Longton and LeGrys (1981) found that addition of pentosans at 1% of the total solids content to 50% starch gels did not significantly alter the kinetics of starch crystallization. This did not agree with the findings of Kim and D'Appolonia (1977a) who showed a significant reduction in the staling process, as measured by firmness, by adding pentosans to starch gels. Therefore, Longton and LeGrys (1981) concluded that DSC and firmness measurements do not record the same process during starch gel ageing. In the present study results from compression measurements of either crumpets or cell-wall material were generally not as well described by an Avrami model as were staling endotherm data. This probably results from the fact that the DSC method is assessing changes in starch which may be expected to proceed in a manner described by the Avrami equation while compression measurements relate to the macrostructure of the product.

5. CONCLUSIONS

The commercial manufacturer of crumpets had found by trial and error that optimum results were obtained in the factory by using a mixture of equal amounts of two flours containing different protein levels and treated differently during production. It was found that the starches from the two flours displayed different characteristics. Starch from Five Roses flour had a higher damage level than that from Primrose flour. Swelling power of Primrose starch was consistently higher than that of Five Roses while their solubilities were essentially equal. Amylose solubility followed the same general form as the total solubility; amylose usually comprised over 50% of the total solubles. Viscosity measurements of dilute starch suspensions showed that Primrose starch and flour had higher apparent viscosities than Five Roses starch and flour. This suggests therefore, that viscosity development under the conditions pertaining to the present study depended more on swelling power than on the existence of a filamentous exudate. Differences between the two starches were probably due to the treatments received during production.

Temperature and heat of gelatinization for the two starches were found to be equivalent. Therefore, degree of gelatinization within the crumpet is not expected to be dependent on starch source, rather, on the temperature history of the product. The main result then, of using a mixture of the two flours is to adjust both the quantity and quality of the protein content in the crumpet. Since Primrose starch swells more than Five Roses starch under the same conditions the range of size of swollen starch granules in the finished product is probably larger than if only one flour were used.

During baking of the crumpet a negative temperature gradient from lower to upper surface is set up. The hot plate is at 200°C while a temperature of ca. 100°C is reached at a position 6 mm above the plate. The upper surface of the crumpet does not achieve temperatures higher than ca. 80°C. The temperature of the central portion of the crumpet increases at a rate of ca. 20 C°/min.

Addition of emulsifiers was not found to have a significant effect on the temperature or heat of gelatinization resulting in no change to the degree of gelatinization in the crumpet over the control. When SSL or MG was added to the batter in a dispersion form they were found to increase the size of the amylose-lipid endotherm. This could be achieved either by promoting complexation of inherent lipids with amylose or by incorporation of the added agent. Addition of emulsifiers in powder form did not show a significant effect on the amylose-lipid endotherm. There was no evidence in the present study of exothermic complexation of added emulsifiers with starch.

Microscopic techniques revealed no changes in crumpet microstructure brought about by emulsifier addition. SEM showed an internal structure of starch granules which, although deformed, were generally discernible. Surfaces of channels and air-holes appeared to be coated either by protein, protein and starch fused together or by highly disrupted starch granules. Light microscopy and selective staining of wall surfaces did not show a protein covering. Therefore, it was concluded that the apparent coat is due mainly to gelatinized starch in a highly disordered state. Protein appeared scattered throughout the product in masses. In some areas, very diffuse networks of protein were seen but these were isolated. Protein masses and starch granules appeared aligned near channels due to forces exerted during baking. The main body of the crumpet appeared to be made up of starch granules, twisted and stretched, probably 'cemented' together by leached-out material. Starch granules at the top surface were hardly swollen and exhibited birefringence; it was only in this region that the bimodal distribution of wheat starch granules could still be observed.

It was found that by heating batter containing emulsifiers to 85°C using a rapid heating rate (20 C°/min) less than maximum possible complexation of amylose and lipid was achieved. This indicates that to obtain a higher degree of complexation, not only must the added emulsifier be in a particular physical state but that the time-temperature regime during baking is also important.

It was found that an Avrami model could not be used to describe starch crystallization at 25°C during a short-term storage trial. Data for storage at 5°C appeared to fit a model with n equal to unity. There was no indication, by these means, that emulsifiers added in powder or dispersion form had any effect on the staling endotherm even though the amylose-lipid endotherm may be increased in size.

X-ray diffraction analysis showed that SSL added in powder form to the crumpet batter resulted in a more intense V-pattern than that of control or MG crumpets, indicating complexation with the starch. However, there was no resultant effect on the size of the 5.2 Å peak.

There was evidence from DSC analysis of a different type of starch crystallization at 5 and 25°C which resulted in the formation of crystallites of a higher degree of perfection at the higher storage temperature.

It may be concluded from the present study that the Avrami analysis should not be used to describe firmness changes in crumpets during storage. Since Avrami kinetics were developed to describe crystallization this is in some agreement with views expressed by Longton and LeGrys (1981) that DSC analysis of the staling endotherm does not measure the same process as compressibility measurements. The Avrami model should be used with caution; estimated parameters, their confidence intervals and relevant statistics should be assessed before using it to compare treatments applied to a subject. It appears more suitable for application to staling endotherm data than to compression data.

Results obtained in the present study provide no evidence that addition of emulsifiers to crumpet batter has an effect in reducing the rate of starch crystallization or the limiting amount of crystallization, or of reducing the firmness of crumpets or crumpet cell-wall material.

Further work is required to develop a model which may be used to quantify changes occurring in the crumpet during storage. A suitable model would facilitate measurement of the effects of various treatments. Other means to delay or reduce staling of crumpets should be investigated, such as addition of other emulsifiers, amylases or humectants.

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7. APPENDIX I -- PREPARATION OF STAINING REAGENTS

7.1 Periodic Acid Schiff (PAS procedure; Gurr, 1962)

7.1.1 Periodate solution

Periodic acid	0.400 g
Distilled water	50.0 ml
Sodium acetate ($CH_3COONa \cdot 3H_2O$)	0.135 g

Ingredients were mixed together at room temperature and stored in an amber bottle. Due to deterioration of this solution after several days, fresh reagent was prepared when required.

7.1.2 Reducing rinse

Potassium iodide	1.00 g
Sodium thiosulphate (pentahydrate)	1.00 g
Distilled water	20.0 ml

The two solids were dissolved in the water, then the following were added with stirring:

98% Ethanol	30.0 ml
2 N Hydrochloric acid	0.5 ml

A precipitate of sulphur formed and was allowed to settle out; this did not affect the efficiency of the reagent. On storage, the reagent tended to lose its acid reaction. Therefore, prior to use, a few drops of 0.5 N HCl were added until the reagent was acidic to litmus.

7.1.3 Schiff's reagent (de Tomasi, 1936)

Basic fuchsin (C.I. 42510)	1.00 g
Distilled water	200 ml
1 N Hydrochloric acid	20.0 ml
Sodium metabisulphite	1.00 g
Activated charcoal	1-2 g

Basic fuchsin was dissolved in distilled water and boiled, after which the vessel was shaken for 5 min and the contents allowed to cool. When the temperature had reached 50°C, the mixture was filtered through Whatman No. 4 filter paper and the hydrochloric acid was added. On further cooling to 25°C, the sodium metabisulphite was added to the vessel which was then stored in the dark at room temperature for 24 h. Then charcoal was added, the mixture shaken well for 1 min and filtered rapidly through Whatman No. 4 filter paper to give a clear, colourless solution. The reagent was stored in a dark bottle at 4°C. Prior to staining, an aliquot was removed from the bottle and allowed to reach room temperature, the unused portion of the aliquot being discarded.

7.1.4 Sulphite wash water

This was used to wash out unbound Schiff's reagent.

1 N Hydrochloric acid	25 ml
Potassium metabisulphite (0.5% w/v aqu. solution)	50 ml

The chemicals were mixed just prior to use.

7.2 Fast Green Protein Stain

A 0.5% w/v aqu. solution of Fast Green FCF (C.I. 42053) was acidified by the addition of 3-4 drops of 1 N sulphuric acid per 50 ml stain solution.

7.3 Gram's Iodine Solution (Flint and Johnson, 1979)

Stock Solution

Iodine	1 g
Potassium iodide	2 g
Distilled water	300 ml

Working Solution

Stock solution	2 ml
Distilled water	8 ml

7.4 Clearing Agent

Xylene Component

Xylene	100 ml
Phenol crystals	5 g

The phenol crystals were added since the xylene forms a whitish emulsion with any residual water in an incompletely dehydrated specimen, which would interfere with the observation of specimen characteristics.

The following series was used in the order given to clear the section:

Mixture	*% Xylene Component*	*% Acetone*
I	25	75
II	50	50
III	75	25
IV	100	0

8. APPENDIK 2: PHYSICOCHEMICAL CHARACTERISTICS OF FLOURS AND STARCHES

Table 8.1 Swelling power at various temperatures of the isolated starches.

Temperature (°C)	Starch Primrose	 Five Roses
60	7.2±0.2[1]	5.7±0.1
65	8.3±0.2	7.6±0.2
69	8.8±0.3	7.7±0.1
74	9.3±0.1	8.4±0.1
80	10.5±0.5	9.2±0.6
85	11.9±0.3	9.8±0.3
89	14.3±1.1	11.2±0.5
95	20.4±0.2	17.6±0.5
97.5	23.4±0.4	19.5±0.6

[1] Mean±S.D.

Table 8.2 Percent solubles at various temperatures of the isolated starches.

Temperature (°C)	Starch Primrose	 Five Roses
60	1.7±0.2[1]	1.7±0.5
65	3.3±0.5	3.5±0.7
69	3.9±1.4	2.9±0.6
74	3.9±0.2	3.8±0.7
80	5.6±0.1	5.2±0.2
85	9.2±0.1	8.1±0.2
89	11.7±0.4	11.7±1.7
95	26.5±0.8	28.4±0.7
97.5	31.6±0.4	33.5±0.7

[1] Mean±S.D.

Table 8.3 Amylose solubility[1].

Temperature (°C)	Starch Primrose	 Five Roses
60	1.4±0.1[2]	1.1±0.1
65	2.3±0.1	2.0±0.1
69	2.9±0.1	2.5±0.1
74	3.6±0.1	3.3±0.0
80	4.9±0.2	4.5±0.1
85	8.7±0.2	7.7±0.2
89	11.4±0.6	10.5±0.6
95	18.4±0.1	20.2±0.9
97.5	22.2±0.1	23.2±0.4

[1] mg amylose in 25 ml aliquot of supernatant.
[2] Mean±S.D.

9. APPENDIX 3: CHARACTERISTICS OF THE STALING ENDOTHERM

Table 9.1 Characteristics of the staling endotherm of control batter in storage trial I.

Storage temp. (°C)	Length of storage (days)	Temperature (°C) T_o	T_p	T_c	Heat of transition (J/g CHO)
5	0	---	---	---	---
	1	40±2	52±2	64.9±0.3	1.5±0.5
	3	42±2	54.6±0.8	64.6±1.1	2.8±0.4
	6	42.5±1.0	54.1±0.8	65.0±1.0	3.4±0.2
25	0	---	---	---	---
	1	---	---	---	---
	3	49.0±1.0	59.7±0.8	66.1±1.0	0.4±0.1
	6	51.5±0.6	62.5±0.9	70.3±1.1	0.6±0.1

Table 9.2 Characteristics of the staling endotherm of SSL batter in storage trial I.

Storage temp. (°C)	Length of storage (days)	Temperature (°C) T_o	T_p	T_c	Heat of transition (J/g CHO)
5	0	---	---	---	---
	1	43.2±1.5	56.6±1.0	65.2±0.8	1.3±0.2
	3	41.2±0.9	53.5±0.6	63.8±0.8	3.2±0.1
	6	41.6±0.6	53.3±0.7	64.0±0.3	4.5±0.3
25	0	---	---	---	---
	1	---	---	---	---
	3	49.2±1.1	59.5±0.3	68.9±1.0	0.4±0.0
	6	51.2±0.7	60.9±1.0	70.2±1.0	0.7±0.2

Table 9.3 Characteristics of the staling endotherm of MG batter in storage trial I.

Storage temp. (°C)	Length of storage (days)	Temperature (°C) T_o	T_p	T_c	Heat of transition (J/g CHO)
5	0	---	---	---	---
	1	41.6±0.8	53.2±0.3	64.1±1.0	1.6±0.1
	3	41.5±1.0	54.3±0.8	63.8±0.8	3.4±0.2
	6	41.6±0.8	54.6±0.6	64.6±0.9	4.1±0.5
25	0	---	---	---	---
	1	---	---	---	---
	3	49.2±1.0	59.5±1.1	67.1±0.6	0.3±0.1
	6	54.3±1.0	61.2±0.6	69.8±0.6	0.5±0.1

Table 9.4 Characteristics of the staling endotherm of control batter in storage trial II.

Storage temp. (°C)	Length of storage (days)	Temperature (°C) T_o	T_p	T_c	Heat of transition (J/g CHO)
5	0	---	---	---	---
	1	42±2	54.7±0.6	64.9±0.6	1.4±0.2
	3	40.1±1.0	53.2±0.9	64.1±0.5	3.2±0.3
	6	41.9±0.0	53.6±0.5	63.5±0.5	4.1±0.4
25	0	---	---	---	---
	1	51±2	58.0±1.0	66±2	0.3±0.1
	3	49.5±0.9	59.6±0.6	66.7±0.3	0.3±0.08
	6	52±2	61.7±0.0	68.6±1.0	0.5±0.3

Table 9.5 Characteristics of the staling endotherm of SSL batter in storage trial II.

Storage temp. (°C)	Length of storage (days)	Temperature (°C) T_o	T_p	T_c	Heat of transition (J/g CHO)
5	0	---	---	---	---
	1	41.3±0.9	54.4±0.8	64.4±0.8	1.2±0.3
	3	41.2±1.0	54.3±0.0	64.7±0.7	2.8±0.4
	6	38.1±1.0	52.1±0.4	63.7±0.3	4.2±0.4
25	0	---	---	---	---
	1	---	---	---	---
	3	54.7±0.6	59±2	64.9±1.4	0.1±0.05
	6	52±2	61.7±0.0	69.1±0.9	0.5±0.2

Table 9.6 Characteristics of the staling endotherm of MG batter in storage trial II.

Storage temp. (°C)	Length of storage (days)	Temperature (°C)			Heat of transition (J/g CHO)
		T_o	T_p	T_c	
5	0	---	---	---	---
	1	42±2	56.6±0.9	66.3±0.4	0.9±0.1
	3	42.2±0.8	54.1±0.8	64.7±0.9	2.6±0.6
	6	39.6±1.0	52.9±0.3	63.5±0.5	4.0±0.3
25	0	---	---	---	---
	1	---	---	---	---
	3	---	---	---	---
	6	49±2	61.2±0.6	69.7±0.9	0.6±0.1